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## ERRATA AND AUTHORS' EMENDATIONS

Page 54, line 2, should read "$F_2$ or $F_4$" instead of "$F_1$ or F."

Page 58, Table V, under last column the first number should read 33 instead of 23.

Page 68, line 24, should read "with a nutrient solution" rather than "with an nutrient solution."

Page 69, lines 9 and 11, should read "O. A. Pratt" instead of "A. C. Pratt."

Page 76, line 3, should read "Plate 11, A" instead of "Plate 11, B."

Page 80 and following, in Plates 10 and 11, figures A and C of Plate 10 should be interchanged with figures A and C of Plate 11.

Page 174, line 1, should read "causal" not "casual."

Pages 175 and 177, and Plates 22 and 24, running head should read, "Transmissible Mosaic Disease of Chinese Cabbage" instead of "Transmissible Mosaic Disease of Cabbage."

Page 178, colored plate is by J. Marion Shull.

Page 242, line 8, should read "Humphrey, as reported by Jones (5)," not "Humphrey (4) states . . . ."

Page 282, line 32, should read "at each treatment" instead of "to each treatment."

Page 283, line 17, should read "nitric acid," not "nitri acid."

v

““# JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXII WASHINGTON, D. C., OCTOBER 1, 1921 NO. 1

## OCCURRENCE OF QUERCETIN IN EMERSON'S BROWN-HUSKED TYPE OF MAIZE

By CHARLES E. SANDO, *Junior Chemist, Office of Physiological and Fermentation Investigations, Bureau of Plant Industry, United States Department of Agriculture*, and H. H. BARTLETT, *Department of Botany, University of Michigan, Collaborator, Office of Physiological and Fermentation Investigations, Bureau of Plant Industry, United States Department of Agriculture*[1]

In connection with the genetical studies of pigmentation in maize which have been carried on for several years at Cornell University by Prof. R. A. Emerson and his students, a plan was made for the writers to collaborate in parallel biochemical studies in the isolation and identification of the pigments from material of known genetical constitution, the latter to be furnished as a by-product of the Cornell experiments.

As a beginning in the work it seemed desirable to undertake a study of the character pair purple versus brown. These are two of the general plant colors recognized in the Cornell experiments, the series running as follows: I, purple; II, sun-red; III, dilute purple; IV, dilute sun-red; V, brown; and VI, green. Full details of the genetic relations of these types are being published by Emerson,[2] to whose memoir the reader should refer for full details. Here it may suffice to say that purple is a color type which is uncommon in cultivation and infrequent in experimental cultures. It is distinguished from other types by the fact that some purple color is developed even in seedlings grown in the dark. At maturity nearly all parts are more or less purple, including the culm, the brace roots, all leaf sheaths, the husks, even the inner ones, the cob, and the staminate inflorescence. In intensity of coloration purple stands at the head of the series of color types. Material of this type was furnished by Prof. Emerson to Dr. John W. Calvin, of the University of Nebraska, before we took over the general problem, and we have therefore confined our attention for the present to the brown type, awaiting a report on his study of the purple type from Dr. Calvin.

[1] In connection with our work with maize we have received numerous favors from Prof. R. A. Emerson and Dr. E. G. Anderson, to whom we tender our best thanks.

[2] EMERSON, R. A. THE GENETIC RELATIONS OF GENERAL PLANT COLORS IN MAIZE. N. Y. Cornell Agr. Exp. Sta. Mem. 39, 156, p., 11 col. pl. 1921.

A few preliminary tests of the purple pigment convinced us that it was an anthocyanin of which the nonsugar portion was of the same general group as cyanidin, isolated by Willstätter [1] and his students from several sources, including the cornflower, Centaurea. His proof of the easy chemical transition from the anthocyanin to the flavonol series led us to look for a member of the latter series in the brown maize. In accordance with expectations, we were able to isolate a glucosid of quercetin. This discovery makes it seem exceedingly likely that the anthocyanin of the purple type is a corresponding glucosid of cyanidin.

The brown color type is a still more unusual one than the purple. It first appeared in Emerson's [2] cultures as a segregate in the second generation of the cross purple × green and is unknown outside this series of cultures. Seedlings and young plants are wholly green. As the flowering period approaches, a brown color appears in the lower sheaths, and at flowering time the culm, sheaths, husks, and staminate inflorescences are brown. Light is not essential to the development of the color. Our material of the brown type consisted of husks.

## ISOLATION AND IDENTIFICATION OF THE FREE QUERCETIN

Ground husks were extracted in a large Soxhlet apparatus with redistilled 95 per cent alcohol for about 72 hours, and the alcohol was evaporated off in vacuo. The thin aqueous sirup was filtered from tarry matter and the filtrate boiled with animal charcoal. By shaking with ether it gave an etherial solution containing a free (nonglucosidal) flavone which proved to be quercetin. The ether was evaporated off, and the residue, after being dried in a desiccator, was extracted in a paper thimble, first with benzene, to remove tarry colloids, oils, etc., and finally, for a short time, with ether. The latter solvent dissolved part of the quercetin but left the bulk of it in the thimble. This portion was dried and acetylated for an hour with anhydrous sodium acetate and acetic anhydrid. After purification the acetyl derivative was quantitatively hydrolyzed with sulphuric acid in glacial acetic acid. The reaction mixture was diluted and the recovered quercetin was washed with cold water. The results are given in Table I.

TABLE I.—*Data on hydrolysis, by sulphuric acid in glacial acetic-acid solution, of the acetylated free flavone of brown maize husks*

| | Sample 1. | Sample 2. | Sample 3. |
|---|---|---|---|
| Weight of acetyl quercetin (gm.) | 0.2521 | 0.3165 | 0.4908 |
| Weight of recovered quercetin (gm.) | .1484 | .1866 | .2902 |
| Percentage of recovered quercetin | 58.86 | 58.95 | 59.13 |

[1] WILLSTÄTTER, Richard, and EVEREST, Arthur E. UNTERSUCHUNGEN ÜBER DIE ANTHOCYANE. I. ÜBER DEN FARBSTOFF DER KORNBLUME. *In* Liebig's Ann. Chem., Bd. 401, Heft 2, p. 189–232, 4 fig. 1913.

[2] EMERSON, R. A. OP. CIT.

The mean of the three determinations is 58.98 per cent—in exact accord with theory.

The entire yield of approximately 1 gm. of acetyl derivative was divided to make the above determinations. The quercetin obtained (0.6254 gm.) was again acetylated, yielding 0.8352 gm. of penta-acetylquercetin. The acetyl derivative melted at 190° to 192° C. The recovered flavone melted at about 305° to 306° with darkening. When mixed with quercetin from Escholtzia (melting point approximately 305° to 310°) the mixture melted at 306° to 307°. In other characteristics the quercetin from maize was identical with a sample obtained by the writers[1] from rutin, a glucosid of quercetin found in Escholtzia petals.

Combustions of the free quercetin and of its acetyl derivative were made, with the results shown in Table II.

TABLE II.—*Combustions of the free quercetin of brown maize husks and of its acetyl derivative*

| | Quercetin. | Penta-acetylquercetin. | |
|---|---|---|---|
| | | Sample 1. | Sample 2. |
| Weight of sample (gm.) | 0.1353 | 0.1126 | 0.2026 |
| Weight of carbon dioxid (gm.) | .2951 | .2430 | .4338 |
| Weight of water (gm.) | .0404 | .0403 | .0691 |
| Percentage of carbon | 59.47 | 58.85 | 58.39 |
| Percentage of hydrogen | 3.34 | 4.00 | 3.82 |

Theory requires: For quercetin, carbon 59.59 per cent, hydrogen 3.34 per cent; for penta-acetylquercetin, carbon 58.59 per cent, hydrogen 3.90 per cent.

## PREPARATION OF THE GLUCOSID

After partition of the alcoholic extract of the brown husks between ether and water, the aqueous solution, containing as one of its chief constituents a quercetin glucosid, was treated with four successive portions of lead acetate. The first fraction of the lead precipitate was discarded. The second consisted largely of tarry matter and was therefore not used for the preparation of pure glucosid but yielded quercetin on hydrolysis after decomposition with hydrogen sulphid. The third and fourth fractions were combined, suspended in hot alcohol, decomposed with hydrogen sulphid, filtered, and evaporated to small bulk. A small quantity of impure glucosid separated out on standing, but the greater part was got by shaking the solution with ethyl acetate.

The glucosid was purified only with great difficulty, by fractional solution of the dry impure product in ethyl acetate and successive crystallization of the purer fractions from water. The yield of pure glucosid

[1] SANDO, Charles E., and BARTLETT, H. H. RUTIN, THE FLAVONE PIGMENT OF ESCHOLTZIA CALIFORNICA CHAM. *In* Jour. Biol. Chem., v. 41, no. 4, p. 495–501, pl. 6–7. 1920.

obtained in this manner was insufficient for a thorough investigation, which must be deferred until a new lot of material is extracted. It was nearest in color to the "lemon yellow" of Ridgway's [1] color standards and melted to a cherry-red liquid at 220° to 222° C. When hydrolyzed it produced quercetin and apparently only one sugar, glucose, although the latter point is to be more thoroughly investigated. The osazone of the sugar melted at 204° to 206° and was evidently glucosazone. The quercetin obtained by hydrolysis was identified by its general properties and by combustions both of the free flavonol and of the acetyl derivative. The latter melted at 191° to 193.5° and had the properties of penta-acetylquercetin. A sample weighing 0.4650 gm. gave 0.2735 gm. of quercetin, or 58.81 per cent by quantitative hydrolysis; theory requires 58.98 per cent. The results of combustions are given in Table III.

TABLE III.—*Combustions of the quercetin obtained by hydrolysis of the glucosid of brown maize husks and of its acetyl derivative*

| | Quercetin. | Penta-acetyl-quercetin. |
|---|---|---|
| Weight of sample (gm.) | 0.1514 | 0.1570 |
| Weight of carbon dioxid (gm.) | .3283 | .3400 |
| Weight of water (gm.) | .0445 | .0537 |
| Percentage of carbon | 59.13 | 59.06 |
| Percentage of hydrogen | 3.29 | 3.83 |

Theory requires: For quercetin, carbon 59.59 per cent, hydrogen 3.34 per cent; for penta-acetylquercetin, carbon 58.59 per cent, hydrogen 3.90 per cent.

The glucosid is not one of the well-known ones but bears considerable similarity to one which Heyl [2] recently isolated from the pollen of ragweed, probably *Ambrosia artemisiifolia* L., although he gives only the common name.

## SUMMARY

In accord with the expectation that the brown-husked type of maize would be found to contain a flavonol, we have been able to isolate from brown husks both free quercetin and a quercetin glucosid of which a further investigation will be made.

The two compounds in question are both lemon yellow in color. If they account for the truly brown color of the husks of this type, it must be through their tinctorial quality, probably through their adsorption on some colloid component of the brown tissues.

It is very probable that the quercetin glucosid is the counterpart in the brown type of the anthocyanin of the purple type. The pigment of the latter will probably be found to be allied to cyanin.

[1] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 pl. (col.) Washington, D. C. 1912.

[2] HEYL, Frederick W. THE YELLOW COLORING SUBSTANCES OF RAGWEED POLLEN. *In* Jour. Amer. Chem. Soc., v. 41, no. 8, p. 1285-1289. 1919.

# BIOLOGICAL ANALYSIS OF THE SEED OF THE GEORGIA VELVET BEAN, STIZOLOBIUM DEERINGIANUM

By BARNETT SURE and J. W. READ, *Laboratory of Agricultural Chemistry, University of Arkansas*

The velvet bean, *Stizolobium deeringianum* Bort., is annually becoming more important in southern agriculture, and the acreage planted to this crop in the cotton belt is continually increasing. From 1915 to 1917 it is estimated that the area increased from less than 1,000,000 acres to more than 5,000,000 acres. The acreage in 1917 was 119 per cent greater than in 1916. It is the most vigorous growing annual legume in the United States; and on account of its very rank growth and the common practice of cultivating it with the corn crop it is chiefly used as a winter pasture for cattle and hogs, although much larger quantities of the beans are harvested from year to year and ground, either with or without the pods, for market purposes. Harvesting with the corn crop for use as silage is also growing in favor.

Because of the rapidly increasing interest in this crop as a feed and its very considerable promise in this respect, particularly to the South, it occurred to one of the authors[1] that a biological analysis should give very fundamental information as to how the velvet bean might best be utilized for feeding purposes. Accordingly the Georgia Velvet Bean, commonly know as the Early Speckled, was chosen for our studies because of its early maturity, general popularity, and adaptability to the more northern as well as to the other sections of the cotton area.

The Georgia velvet bean seed has a very tough, hard hull which constitutes 12 per cent of the whole seed. In grinding the beans it was found impossible to grind the hulls in a satisfactory manner; consequently these were sifted out. The experiments reported in this paper were conducted with hulled seed. It was later found, however, that after the sifted hulls had been dried on a steam bath for from six to eight hours they could be ground; and experiments were later inaugurated, introducing the hulls in the same proportions as they were found to exist in the seed, the results of which will be reported later, together with other data showing the supplementary relationships of the seed to the leaf and the biological value of the whole plant. The nutritive value of the seed and the whole plant in practical rations is also being studied at the present time.

[1] Credit for the inauguration and outline of the velvet-been studies as approved under the Adams Fund is due Prof. J. W. Read.

The experiments reported in this paper were conducted with albino rats, employing the standard technic adopted by the Department of Agricultural Chemistry of the University of Wisconsin.

Preliminary experiments showed that young rats, 40 to 90 gm. in weight, will exist only from 7 to 12 days on a diet composed solely of

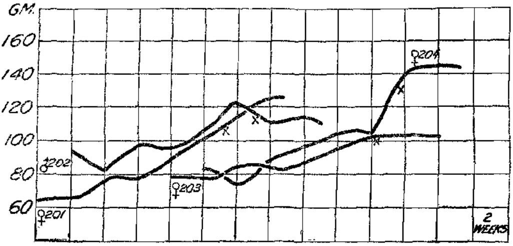



FIG. 1.—Gain in weight of lot 51 on ration of velvet beans, 80 per cent; butter fat, 5 per cent; No. 32 salts, 4 per cent; and dextrin, 11 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo. At point x 9 per cent of the velvet beans was replaced by 9 per cent casein.

the raw hull-less velvet bean seed and a liberal supply of distilled water. The food consumption could be increased 50 to 60 per cent, however, by feeding young animals the seed after it was autoclaved for one hour at 15 pounds pressure. Consequently, autoclaved velvet beans were

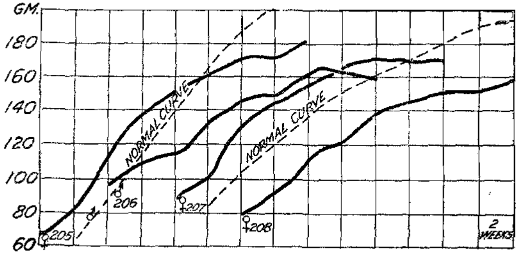



FIG. 2.—Gain in weight of lot 52 on ration of velvet beans, 60 per cent; butter fat, 5 per cent; No. 32 salts, 4 per cent; casein, 5 per cent; and dextrin, 26 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo.

used in all this work. In order to determine whether the water-soluble vitamin was destroyed in the process of autoclaving, controls were run with uncooked beans.

Even when fed upon autoclaved beans, animals, although consuming considerably more of the seed, existed only for a period of 17 to 21

days when the diet was composed solely of the seed. On diets composed of 80 per cent velvet bean with 20 per cent dextrin, and 60 per cent velvet bean with 40 per cent dextrin, eight animals, having an initial weight of 50 to 60 gm., maintained their weight for a period of eight weeks but made no growth. Likewise, when the velvet bean formed 60 to 40 per cent of the ration, respectively, as a source of protein, in the presence of all the other dietary factors, no growth resulted, but all the animals maintained their body weight for a period of six weeks, indicating that the proteins in the seed are deficient.

Since these experiments lasted for periods ranging from six to eight weeks only, no charts were prepared illustrating the points mentioned. All the rest of our findings are illustrated in figures 1 to 15.

When velvet beans formed 80 per cent as the source of protein in the ration (fig. 1), very little growth resulted. Although at point *x* 9 per cent of the beans was replaced by 9 per cent casein, no appreciable change in the character of growth ensued, lack of response to the addition of purified casein being due, as it will be noted from the following graphs, to the injurious effect of this high plane of velvet bean intake.

Velvet beans fed at a 60 per cent level as a source of protein, supplemented with 5 per cent casein, produced a fair amount of growth (fig. 2).

When, however, 40 per cent velvet beans was the source of protein and the ration was further fortified with 9 per cent casein, the two females made normal growth for a period of four months and the two males grew at a rate even beyond the expectation curve (fig. 3). Rat 211 was unable to rear her young, although her litter was reduced from nine to four.

Figure 4 shows that young animals are unable to make any growth on a ration composed of 80 per cent velvet beans as the source of salts.

When 40 per cent velvet beans served as the source of salts, some little growth occurred during the first 10 weeks (fig. 5). It is evident, then, that at least part of the failure of lot 61 (fig. 4) must be ascribed to the harmful effect of the higher plane of velvet bean intake. A striking change in the character of growth is apparent when at point *x* 4 per cent of dextrin was replaced by 4 per cent of salt mixture No. 32.[1]

When 1 per cent sodium chlorid (NaCl) and 1.5 per cent calcium carbonate ($CaCO_3$) replaced salt mixture No. 32 in the ration, very good growth was obtained for a period of three months (fig. 6), indicating that the calcium, sodium, and chlorid ions furnish the necessary mineral supplements in the velvet bean seed.

Figure 7 shows that when 1.5 per cent calcium carbonate alone replaces salt mixture No. 32 only a little growth results.

[1] Steenbock, H., and Gross, E. G. FAT SOLUBLE VITAMINE. II. THE FAT-SOLUBLE VITAMINE CONTENT OF ROOTS TOGETHER WITH SOME OBSERVATIONS ON THEIR WATER-SOLUBLE VITAMINE CONTENT. *In*

Figures 1 to 7 have indicated that velvet beans are detrimental to young experimental animals when fed at an 80 per cent level. The experiment on which figure 8 is based corroborates that fact. Although

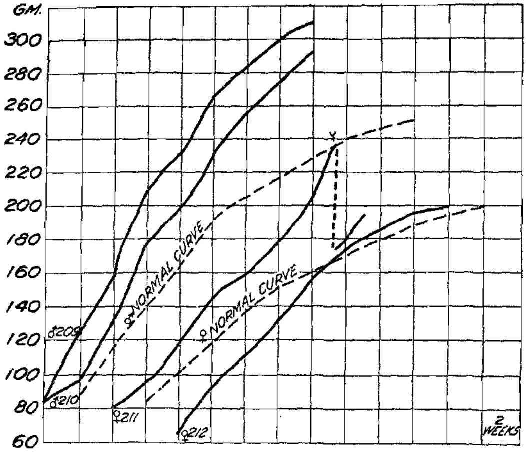



FIG. 3.—Gain in weight of lot 53 on ration of velvet beans, 40 per cent; butter fat, 5 per cent; No. 32 salts, 4 per cent; casein, 9 per cent; and dextrin, 42 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo. *Y* indicates point at which young were littered.

when 80 per cent velvet beans served as a source of the fat-soluble vitamin, two animals made a fair amount of growth for a period of four

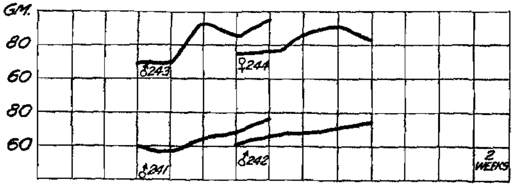



FIG. 4.—Gain in weight of lot 61 on ration of velvet beans, 80 per cent; butter fat, 5 per cent; casein, 5 per cent ;and dextrin, 10 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo.

months; two rats died after six weeks. The failure of these two animals can not be ascribed to the low concentration of the fat-soluble vitamin in the seed, since lot 70 (fig. 11) made normal growth for a period

of over five months when only 20 per cent velvet beans served as a source of that vitamin.

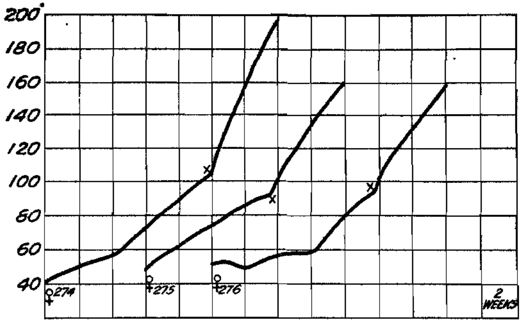



Fig. 5.—Gain in weight of lot 69 on ration of velvet beans, 40 per cent; butter fat, 5 per cent; casein, 9 per cent; and dextrin, 46 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo. At point *x* 4 per cent dextrin was replaced by 4 per cent No. 32 salts.

A considerable improvement in the character of growth is obtained when the plane of intake of velvet beans used to supply the fat-soluble vitamin is reduced from 80 to 60 per cent (fig. 9).

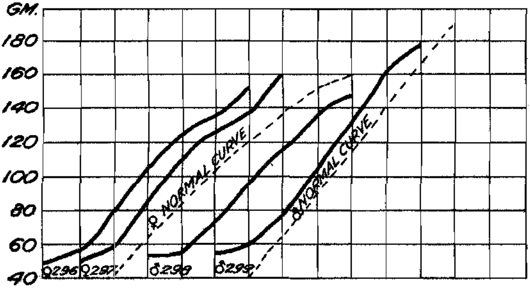



Fig. 6.—Gain in weight of lot 75 on ration of velvet beans, 40 per cent; butter fat, 5 per cent; sodium chlorid, 1 per cent; clacium carbonate, 1.5 per cent; casein, 9 per cent; and dextrin, 43.5 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo.

When the level of velvet bean intake was reduced to 40 per cent, normal growth was obtained. Rat 270 failed to rear her young, although her litter was reduced from eight to four (fig. 10).

Even when the seed was reduced to as low a plane of intake as 20 per cent, it served as a very efficient carrier of the fat-soluble vitamin. It is also apparent that autoclaving for one hour at 15 pounds pressure had

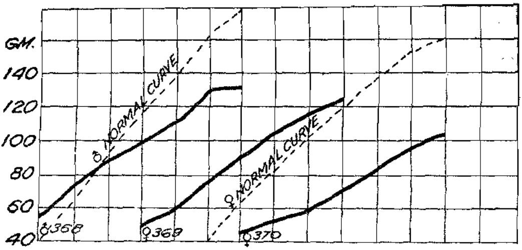



FIG. 7.—Gain in weight of lot 92 on ration of velvet beans, 40 per cent; butter fat, 5 per cent; calcium carbonate, 1.5 per cent; casein, 9 per cent; and dextrin, 44.5 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo.

no deleterious effect on this vitamin. Although excellent growth was obtained on this ration, mother rats No. 277 and 278 failed to rear their young in every case even when their litters ranging from 7 to 10 were reduced to only 4 (fig. 11).

FIG. 8.—Gain in weight of lot 63 on ration of velvet beans, 80 per cent; No. 32 salts, 4 per cent; casein, 5 per cent; and dextrin, 11 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted embryo. D indicates point at which rat died.

Figure 12 shows that on reducing the level of velvet bean intake to 10 per cent as a source of fat-soluble vitamin the character of growth is considerably impaired.

When 80 per cent velvet beans was used to supply the water-soluble vitamin very little growth resulted, one animal dying after three weeks on this ration (fig. 13).

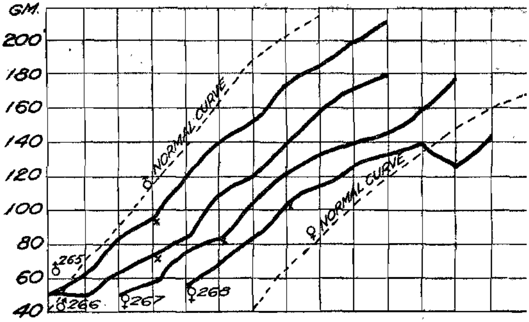
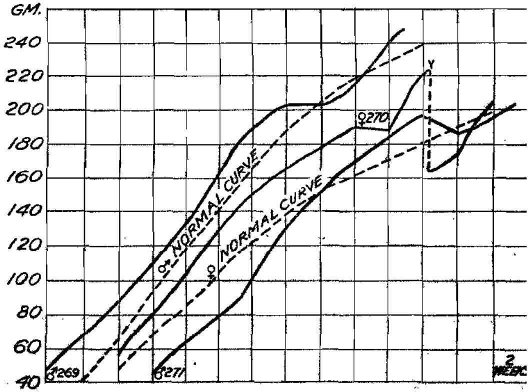



Fig. 9.—Gain in weight of lot 67 on ration of velvet beans, 60 per cent; No. 32 salts, 4 per cent; case 5 per cent; and dextrin, 31 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extrac wheat embryo. At point x 4 per cent dextrin was replaced by 4 per cent additional casein.



Sixty per cent of velvet beans used to supply the water-soluble vitamin allowed only a very small amount of growth (fig. 14).

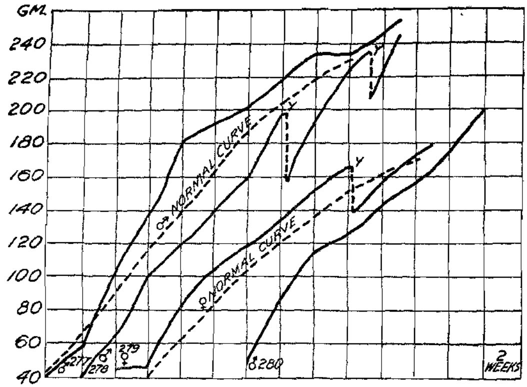



FIG. 11.—Gain in weight of lot 70 on ration of velvet beans, 20 per cent; No. 32 salts, 4 per cent; casein, 12 per cent; and dextrin, 64 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo. *Y* indicates point at which young were littered.

In order to determine whether the water-soluble vitamin was destroyed during the process of autoclaving, 40 per cent velvet beans was fed uncooked. *Very little growth resulted, nor was there any improvement*

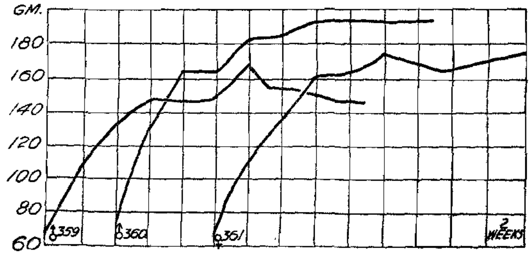



FIG. 12.—Gain in weight of lot 90 on ration of velvet beans, 10 per cent; No. 32 salts, 4 per cent; casein, 16 per cent; and dextrin, 70 per cent. The dextrin carried alcoholic extract of 15 gm. ether-extracted wheat embryo.

in the character of growth when, at point *x*, 10 per cent dextrin was replaced with 10 per cent of an alcoholic extract of ether-extracted wheat embryo. It will be noted that after point *x* this ration is identical with

that given to lot 53 (fig. 3) with the exception that lot 86 received the beans raw while lot 53 received the beans cooked. The striking difference in the character of growth obtained in these two experiments must be attributed to the fact that the velevt bean seed uncooked is either toxic or indigestible at a concentration as low as 40 per cent. The

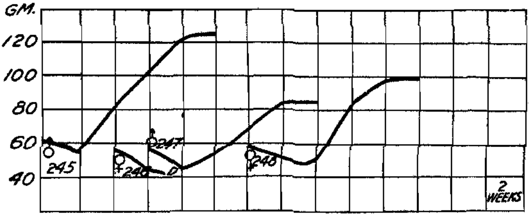



FIG. 13.—Gain in weight of lot 62 on ration of velvet beans, 80 per cent; butter fat, 5 per cent; No. 32 salts, 4 per cent; casein, 5 per cent; and dextrin, 6 per cent. *D* indicates point at which rat died.

cause of the deleterious effect of the raw seed is being studied and will be reported later.

## DISCUSSION

The Georgia velvet bean, Early Speckled variety, has been found to be injurious when fed in the raw condition at as low a level as 40 per cent intake. This has been evidenced from an experiment where 40 per cent

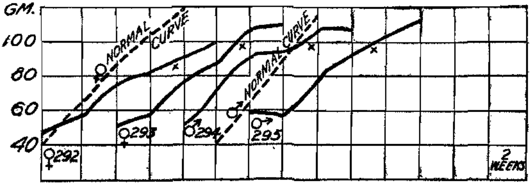



FIG. 14.—Gain in weight of lot 74 on ration of velvet beans, 60 per cent; butter fat, 5 per cent; No. 32 salts, 4 per cent; casein, 5 per cent; and dextrin, 26 per cent. At point *x* 4 per cent dextrin was replaced by 4 per cent additional casein.

velvet beans uncooked formed the source of water-soluble vitamin (fig. 15). Growth was inhibited during the first six weeks of experimentation, after which time 10 per cent dextrin was replaced by an alcoholic extract of 10 gm. ether-extracted wheat embryo. This addition of the water-soluble vitamin should have rendered the ration entirely satisfactory, judging by the character of growth obtained in a duplicate experiment where the beans were furnished cooked (fig. 3).

The nature of the possible toxicity of the velvet bean has been recently suggested by Miller [1] to be due to dihydroxypehnylalanine.

Cooking the seed at 15 pounds pressure for one hour destroyed for the most part its harmful effects, but there was still some injury when fed cooked at as high a plane of intake as 80 per cent. When 80 per cent of the velvet bean served as a source of protein, little growth resulted, nor was there a response obtained after 9 per cent of the seed was replaced by 9 per cent of casein, although when only 40 per cent velvet bean was served as a source of protein, supplemented with the same amount of casein, excellent growth was obtained. Unpublished data in this laboratory show that the better growth on the lower level of seed intake is not to be attributed to the higher intake of dextrin. Additional evidence is apparent from the fat-soluble vitamin experiment that when cooked

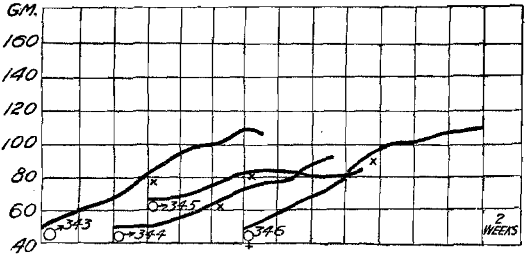



FIG. 15.—Gain in weight of lot 86 on ration of velvet beans (uncooked) 40 per cent; butter fat, 5 per cent; No. 32 salts, 4 per cent; casein, 9 per cent; and dextrid, 42 per cent. At point x 10 per cent dextrin was replaced by 10 per cent of an alcoholic extract of ether-extracted wheat embryo.

velvet beans are fed at an 80 per cent level some injury is still produced. Reduction of the plane of intake from 80 to 40 per cent results in considerable improvement in growth.

That the velvet bean seed is very rich in the fat-soluble vitamin is evident from the fact that normal growth was obtained for a period of over five months when only 20 per cent of the seed served as the source of this syndrome. Reduction of the plane of velvet-bean intake to 10 per cent resulted in inferior growth. The fact that considerably inferior growth was obtained on lower levels of seed intake with larger amounts of casein and dextrin precludes, we believe, the possibility that our casein and dextrin might have furnished appreciable amounts of the fat-soluble vitamin at the higher levels of seed intake, where we had remarkable success. It is also apparent from these experiments that autoclaving the seed for one hour at 15 pounds pressure has no deleterious effect on the fat-soluble vitamin.

[1] MILLER, Emerson R. DIHYDROXYPHENYLALANINE, A CONSTITUENT OF THE VELVET BEAN. *In* Jour. Biol. Chem., v. 44, no. 2, p. 481–486. 1920.

The hull-less seed contained 27.5 per cent protein and therefore furnished 16.5 per cent protein when fed at a 60 per cent level; however, this amount of protein was inadequate for growth even though all the other factors in the diet were rendered satisfactory by the addition of isolated purified food substances.

Recently Johns and Waterman [1] have isolated two globulins and an albumin from the Georgia velvet bean and have reported analytical data on their composition, using the Van Slyke [2] method of protein analysis. Their results show that, with the exception of the albumin, which is low in histidin, the three proteins of the Georgia velvet bean are quite satisfactory for their diamino-acid content. However, since we have insufficient chemical data on the amino-acid content of the Georgia velvet bean no correlation can be made at present between the chemical composition and the biological response of this seed. The nature of the amino-acid deficiencies is being investigated.

The velvet-bean seed has also been found to be deficient for growth in the character of its salts; however, sodium chlorid and calcium carbonate seemed to replace salt mixture No. 32 satisfactorily.

The concentration of the water-soluble vitamin in the seed has been found to be low. Unpublished data show that the addition of the ground hulls in the same proportions as they occur in the whole seed does not improve the water-soluble vitamin content. It is not apparent from these experiments whether this vitamin was in any way destroyed during the process of autoclaving, since the seed was extremely injurious when fed uncooked.


SUMMARY

(1) The Georgia velvet bean seed, Early Speckled variety, when fed raw was found injurious to young rats even when constituting only 40 per cent of the total ration.

(2) Autoclaving the seed for one hour at 15 pounds pressure destroys most of this injury, so that it is possible to include 60 per cent of the bean in a ration. A ration composed of 80 per cent velvet bean cooked still shows some harmful effects.

(3) This seed, unlike most seeds so far studied biologically, is very abundant in the fat-soluble vitamin. The fat-soluble vitamin as it exists in this seed is quite stable after the seed is autoclaved for one hour at 15 pounds pressure. The water-soluble vitamin, however, is of low concentration in the hulled seed.

(4) Both the proteins and salts of the velvet bean have been found to be of deficient character for growth.


[1] JOHNS, Carl O., and WATERMAN, Henry C. SOME PROTEINS FROM THE GEORGIA VELVET BEAN, STIZOLOBIUM DEERINGIANUM. *In* Jour. Biol. Chem., v. 42, no. 1, p. 59-69.

[2] VAN SLYKE, Donald D. THE ANALYSIS OF PROTEINS BY DETERMINATION OF THE CHEMICAL GROUPS CHARACTERISTIC OF THE DIFFERENT AMINO-ACIDS. *In* Jour. Biol. Chem., v. 10, no. 1, p. 15-55, 2 fig. 1911.

# EFFECT OF SOIL TEMPERATURE UPON THE DEVELOPMENT OF NODULES ON THE ROOTS OF CERTAIN LEGUMES

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## INTRODUCTION

During a search for the cause of a diseased condition of alfalfa under observation in 1917 and 1918 the senior writer was led by observations contributed by H. L. Westover, of the Office of Forage Crop Investigations, to believe it likely that soil temperature, within the range which occurs in cultivated fields, affects the initiation and the development of nodules on the roots of alfalfa and perhaps all other legumes to such a degree that the assimilation of nitrogen by these plants is greatly modified by this factor during the summer. The probable importance of such an effect of temperature, should it be demonstrated, upon the development of alfalfa and other legumes and especially its possible relation to the disease in question seemed adequate reasons for making a beginning at the experimental determination of the facts. Experimental methods suitable for the performance of this work had already been highly developed in the course of the study of soil-inhabiting plant parasites at the University of Wisconsin. Thus it came about that the collection of the following data was begun at Madison by the junior author in 1917 and continued by both authors in 1919 and 1920. A temporary suspension of the work is the immediate reason for the publication of this preliminary report.

In the beginning, interest was centered upon ascertaining to what extent soil temperature determined the number of nodules which any of a selected group of legumes might develop. Later the size and composition of the nudoles appeared more significant than the number. Finally, it is seen that soil temperature probably affects profoundly the rate of nitrogen fixation within the nodules of the legumes studied and its assimilation by the plants. A complete demonstration of such an effect and a quantitative determination of its amount remains for the future. During the progress of the work certain striking effects of soil temperature upon the development of the plants quite apart from any relation to nodule formation have been noted.

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## ENVIRONMENTAL FACTORS WHICH HAVE PREVIOUSLY BEEN FOUND TO MODIFY THE FORMATION AND DEVELOPMENT OF NODULES

In the extensive studies which have been made of the conditions which may favor or hinder the development of nodules, no one, so far as the writers are aware, has concerned himself with the factor which is considered here—namely, the temperature of the soil. There are a number of factors, however, which are known to have very much influence upon nodule development, and two of these which may have become modified by our experimental methods for controlling soil temperature must be considered. These are soil moisture and the concentration of nitrates.

With regard to soil moisture, there appears to be both observational and experimental data which indicate that high soil moisture tends to increase nodule formation. Gain [1] notes that peas grown in wet soil have far more nodules than those grown in drier soil close by. Wilson [2] in his experimental work reports that wet soil induces the formation of a greater number of nodules on soybeans. Fortunately, in experimental work with controlled temperatures, it is comparatively easy to maintain soil moisture at a predetermined point with very slight fluctuation. In the preliminary experiments, although no attempt was made to control soil moisture exactly, it is not believed to have fluctuated sufficiently to affect results appreciably. In the later work, soil moisture was maintained in each series at one-half the moisture-holding capacity of the soil used (14 per cent of the wet weight) by weighing the pots each day, if necessary, and restoring the water lost by evaporation and transpiration. It is believed that this method kept variation in soil moisture within such very narrow limits that this factor could not have produced appreciable variation in nodule formation.

That the amount of nitrate present in the soil affects nodule development, completely inhibiting it when high concentrations have been reached, has been demonstrated by several investigators. Wilson [2] has added a considerable number of nitrates to soils in different amounts to determine the concentration at which nodule formation is inhibited by each of the compounds. Although complete inhibition is effected only at concentrations which are not likely to occur in normal soils, the marked effect of variations is so well attested that any differences arising unavoidably during an experimental series must be taken into account in the consideration of results.

The control of the concentration of nitrates in the soil solution in a soil held at different temperatures offers difficulties which can be over-

[1] GAIN, Edmond. INFLUENCE DE L'HUMIDITÉ SUR LE DÉVELOPPEMENT DES NODOSITÉS DES LÉGUMINEUSES. *In* Compt. Rend. Acad. Sci. [Paris], t. 116, no. 24, p. 1394–1397. 1893.

[2] WILSON, J. K. PHYSIOLOGICAL STUDIES OF BACILLUS RADICICOLA OF SOY BEAN (SOJA MAX PIPER) AND OF FACTORS INFLUENCING NODULE PRODUCTION. N. Y. Cornell Agr. Exp. Sta. Bul. 386, p. 363–413, fig. 80–94. 1917.

come only within certain limits. As was expected in advance, the rate of nitrification in soil differs greatly at the different temperatures, producing greatly different concentrations within a short time after a series of plants have been started. In addition, there is soon considerable difference in the size of the plants at the different temperatures and a consequent difference in ability to absorb nitrates. The variations which arise from these causes can be limited somewhat by the use of soil low in total nitrogen, thus making impossible the accumulation of large amounts of nitrates in any case. Variations in the concentration of nitrates which have been observed in the experimental work described here will be noted later, and their possible effect upon the results will be discussed.

## APPARATUS AND METHODS

The apparatus used for the control of soil temperature in these experiments is that which has been used in the Laboratory of Plant Pathology at the University of Wisconsin for several years and needs no new description.[1] In all cases plants were grown in metal cans 6 inches in diameter and 10 inches deep. The number of plants which could be grown in each can without serious crowding of roots was 3 for soybeans, 5 for peas, and 10 for clover and alfalfa. The soil used was a sandy loam from a pasture which had never been cultivated. To this was added about an equal weight of sand in order that the total nitrate content should be kept low and that the mechanical condition of the soil should permit the easy removal of the roots. The temperatures noted in the different series were those at which the water was maintained in the tanks in which the cans were set. Fluctuations of temperature did not often exceed 1° C. from those given in the tables, and were of only a few hours' duration. Although record was made twice daily of the actual temperatures, it is not believed that a computation of the mean temperature from these figures would give a figure more significant than the convenient even numbers used here. It should also be noted that although the surface of the soil was insulated to some degree from loss of heat and moisture by the use of mineral wool, nevertheless at the higher temperatures the surface soil to the depth of about 1 inch was usually cooler by 1 to 1½° than the water. However, it is believed that the larger part of the roots and nearly all the nodules were sufficiently deep in the soil below this cooler layer, so that error arising from this source is not considerable.

Water was supplied through a glass tube which passed to the bottom of the metal can where it entered an inverted unglazed flower pot 3 inches in diameter, which acted as a reservoir. In the last series the

[1] JONES, L. R. SOIL TEMPERATURES AS A FACTOR IN PHYTOPATHOLOGY. *In* Plant World, v. 20, no. 8, p. 229–237, 2 fig. 1917. Literature cited, p. 236–237.

inverted pot was placed about 3 inches below the surface instead of at the bottom in the hope of maintaining a more uniform and rapid distribution of moisture. This appears to have been an unfortunate change in method, since at the higher temperatures roots tended to collect around these pots, where they apparently developed more extensively and produced more nodules than they had in the previous series. Inasmuch as no accumulation of roots took place at lower temperatures, it is not easy to explain this fact.

Attention should here be drawn to the fact that two distinctly different methods of securing data have been used. In the preliminary experiments, the plants were first grown in the cans at ordinary greenhouse temperature for about two weeks before inoculation was made, with the suitable strain of *Bacillus radicicola* Beyr., by pouring a water suspension of the organism around the base of the plants, and the cans were placed in the tanks adjusted at the predetermined temperatures. In the later series the plants were grown from seed in inoculated soil held at the required temperatures from the beginning. Several reasons led to the change of method. In the first place, one could not be certain that the bacteria poured around the plant in the first instance would become rapidly distributed through the soil at all temperatures. This inequality in rate of distribution might affect the number of infections and hence the number of nodules formed. At least it might tend to limit the formation of nodules to the roots near the surface of the ground where temperature is less exactly controlled. A second objection to this method appeared when the marked effect of temperature upon the morphology of the roots themselves was observed. The number of root hairs through which infection has been found to take place is much greater at lower temperatures than at higher. In view of the possible effect of this difference it appeared preferable to grow the plants from the beginning in inoculated soil at the designated temperatures, even though the plants thus produced would necessarily vary considerably in size. Data obtained by each of these methods will be presented.

## MEASUREMENT OF EFFECT OF SOIL TEMPERATURE UPON NODULE FORMATION

When the experiments were begun it was assumed that different temperatures, if they are at all potent, would produce such a marked effect upon the number of nodules that count alone would give a significant expression of results. This expectation was fostered by the fact that Wilson [1] and nearly all previous investigators have used numbers to express similar experimental results. It will be seen from data given later that this hope was early disappointed. Different temperatures usually seemed to affect number not nearly so much as rate of develop-

[1] WILSON, J. K. OP. CIT.

ment and size. In fact, in some instances, volume of nodular tissue seemed in inverse ratio to number. With most legumes it is not easy to get an accurate dry-weight determination of small nodules, because these occur as swellings so closely attached to the root that it is hardly feasible to separate them from the true root tissue. The one species tried which gave least trouble from this source by reason of the distinct separation of its nodules from the root, even at early stages of development, was the soybean. For this reason, it alone was used in the final series recorded here.

Of course it was soon realized during the progress of the work that volume of production of nodules was only an easily observed index, significant chiefly in so far as it revealed important effects of temperature upon the physiological processes which are dependent upon the nodular structures. It would be of greater interest, for example, to measure the amount of nitrogen fixed in these nodules produced at different temperatures and that portion which becomes available to the plant for use in its vital processes. The demonstration of an important limiting effect of temperature upon nitrogen fixation would be of no inconsiderable importance. Such an effect would probably be indicated by large differences in size of nodules, though it might occur without the appearance of such difference. In any case it seems easily possible to determine approximately the efficiency of nodules in the fixation of atmospheric nitrogen by growing parallel series of plants, inoculated and uninoculated, in the same kind of soil and at the same soil temperatures. If the amount of nitrogen in the inoculated plants (aside from that found in the nodules on those plants) is greater than in the uninoculated, the gain must be credited to the efficiency of the nodules. The gains thus found should be an accurate measure of the effect of soil temperature upon the fixation of available nitrogen in the legume used in the experiment, and a comparison of this gain with the weights of the nodules found on the inoculated plants should give an approximate idea of the relation existing between efficiency of fixation of available nitrogen and volume of nodules. In the last series recorded an attempt was made to carry out this experiment with the soybean plant. Unfortunately some of the uninoculated plants in the series became inoculated during the experiment and developed a few nodules, thus making it necessary to discard the data so far as these controls are concerned. Thus an exact determination of the extent of the effect of soil temperature upon the fixation of nitrogen in the nodules of legumes remains to be made. For the present we can only ascertain the dry weights of the nodules themselves as they are found at the end of a period of time and determine the amount of nitrogen found within them.

## DISCUSSION OF THE LEGUMES USED AND THEIR BEHAVIOR UNDER THESE EXPERIMENTAL CONDITIONS

Four legumes were selected for the soil temperature series requiring four different strains of *Bacillus radicicola* for their inoculation. One of these, the soybean, flourishes well at high soil temperatures; one, the Canada field pea, requires a low soil temperature for good growth; and red clover and alfalfa occupy intermediate positions.

A few of the more striking reactions of the plants in these series will be noted. The Canada field pea does not flourish vigorously at a soil temperature as high as 30° C. (Table I) and is intolerant of temperatures above this point, maintaining roots only very close to the surface of the soil. It is perhaps misleading to infer that the lower surface temperature is alone responsible for the position of the roots, since in field plots in hot, exposed positions the death of deeper roots and the formation of surface roots has been noted in hot weather.

Perhaps the more striking effect of the series of soil temperatures upon the soybean plants, aside from the fact of the wide range through which it grows vigorously, is the effect upon the color of the foliage. After the plants had become 5 or 6 inches tall, in both series the leaf color was much darker at the two ends of the series, especially at 30° C. and above, than at 21° and 24°. This difference persisted, tending rather to increase as long as the plants were grown.

One striking difference in behavior between peas and soybeans on the one hand and clover and alfalfa on the other was noted in this series. The annuals formed a rather regular series of plants as judged by appearance (Pl. 1) and also by dry weights (Table I). But with alfalfa and red clover the seedlings at the lower temperatures, 12° and 15° C., though little delayed in starting, remained small Alpine plants with thick dark green leaves and with much red color in the petioles; whereas at 18° the plants were more nearly what may be termed "normal" plants, larger, with fairly long petioles containing less red color.[1]

## EXPERIMENTAL DATA

### EFFECT OF SOIL TEMPERATURE UPON THE NUMBER OF NODULES FORMED

As previously noted, in the first two preliminary trials the seeds were planted in soil in the metal cans and grown for about 10 days at greenhouse temperature (about 22° to 23° C.) before they were inoculated with the suitable strains of *Bacillus radicicola* and placed in the tanks adjusted at the temperatures designated. When it was believed that sufficient time had elapsed for infection at all temperatures, the tops were cut from the plants, dried, and weighed. The roots were carefully

[1] Since this was written clover and alfalfa have been grown under similar conditions at controlled temperatures. The marked dwarfing of plants at 15° and 12° C. was found to disappear when the plants became older, and especially later in the spring when light intensity became greater.

washed from the soil and the nodules were counted. The count obtained is given in Table I. In the two later series the seeds were planted in soil which had already been placed in the tanks adjusted to the temperatures designated. The air temperature ranged from 14° to 18°. The counts obtained in these series are given in Table II.

TABLE I.—*Average number of nodules produced on plants 26 days old grown at a soil temperature of about 20° C. for 10 days, after which inoculation was made and the temperature of the soil was maintained as indicated*

| Temperature. | Alfalfa. | | Red clover. | | Soybeans. | | Field peas. | |
|---|---|---|---|---|---|---|---|---|
| | 20 plants. | 20 plants. | 20 plants. | 20 plants. | 6 plants. | 6 plants. | 10 plants. | 10 plants. |
| °C. | | | | | | | | |
| 10 to 12 | 8 | 4 | 17 | 7 | 0 | 0 | 27 | 21 |
| 15 | 15 | 18 | 27 | 16 | 0 | 0 | 31 | 24 |
| 20 | 35 | 19 | 40 | 47 | 46 | 14 | 37 | 30 |
| 25 | 18 | 23 | 69 | 4 | 61 | 25 | 43 | 60 |
| 30 | 16 | 16 | 75 | 75 | 37 | 33 | 128 | 64 |
| 35 | 5 | 4 | 4 | 6 | 35 | 28 | 0 | 3 |
| 40 | 0 | 1 | (a) | (a) | 0 | 0 | ...... | ...... |

a The plants did not survive.

TABLE II.—*Average number of nodules produced on plants grown at the soil temperatures designated*

| Temperature. | Alfalfa. | Red clover. | Soybeans. | | Field peas. | |
|---|---|---|---|---|---|---|
| | 20 plants 63 days old. | 10 plants 63 days old. | 6 plants 63 days old. | 9 plants 55 days old.[a] | 5 plants 32 days old. | 5 plants 52 days old. |
| °C. | | | | | | |
| 12 | 1.19 | 1.8 | 0.0 | ......... | 3.6 | 2.6 |
| 15 | 1.6 | 5.0 | 6.1 | 19.3 | 3.6 | 9.0 |
| 18 | 16.7 | 12.5 | 5.9 | 13.0 | 8.0 | 27.2 |
| 21 | 8.0 | 24.6 | 4.0 | 14.7 | 14.0 | 23.8 |
| 24 | 3.4 | 17.4 | 11.3 | 19.7 | 25.8 | 13.0 |
| 27 | 11.6 | 11.5 | 8.0 | 18.8 | 30.0 | 58.0 |
| 30 | 10.0 | 8.9 | 5.8 | 16.4 | 4.0 | ......... |
| 33 | 10.7 | 5.3 | 8.8 | 20.0 | ......... | ......... |
| 36 | 3.5 | 0.0 | 13.6 | 12.4 | ......... | ......... |

a The larger number of nodules on the plants 55 days old as compared with those on plants 63 days old is believed to be due largely to the fact that this series of plants was grown in spring, when longer days promoted a far more vigorous growth than was produced by the other plants, which were grown in winter.

Although it will be seen at once that the data in the two tables are not strictly comparable, nevertheless some temperature effects upon number appear. Most conspicuous of all is the greatly increased number upon peas near the upper thermal limit. But this increased number is accompanied by a more than proportionate decease in size. No plant has been found to produce large nodules at 30° C. or above. Clover and alfalfa tend to produce their largest numbers of nodules in the middle portion of the range. Soybeans show no decided temperature effect at all, so

far as number is concerned. However unsatisfactory these figures may be from several points of view, nevertheless they establish one important fact beyond reasonable doubt: Modified soil temperature within the range which these plants can be expected to encounter in the field and even within which they can be grown with vigor under experimental conditions does not prevent the infection of roots by *Bacillus radicicola* and the formation of considerable numbers of nodules. In other words, *B. radicicola*, considered as a parasite, does not show the strongly marked inhibition of its ability to infect roots of plants that has been found in a number of fungus parasites.[1]

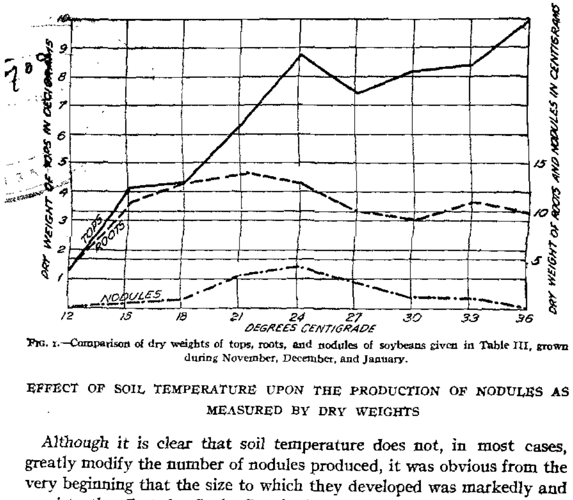



FIG. 1.—Comparison of dry weights of tops, roots, and nodules of soybeans given in Table III, grown during November, December, and January.

## EFFECT OF SOIL TEMPERATURE UPON THE PRODUCTION OF NODULES AS MEASURED BY DRY WEIGHTS

Although it is clear that soil temperature does not, in most cases, greatly modify the number of nodules produced, it was obvious from the very beginning that the size to which they developed was markedly and consistently affected. Such effect is shown graphically in Plate 2, where nodules from an equal number of plants are shown placed in rows and in Plate 3, where nodules from a larger number of plants are placed in tubes of equal diameter. Dry weights of the nodules shown are given in Table III. Data are given for the soybeans only for reasons already mentioned, but, judging by visual evidence obtained in studying all four legumes used, it is believed that all behaved in essentially the same manner.

[1] JOHNSON, James, and HARTMAN, R. E., INFLUENCE OF SOIL ENVIRONMENT ON THE ROOT-ROT OF TOBACCO. *In* Jour. Agr. Research, v. 17, no. 2, p. 41–86, pl. 1–8. 1919. Literature cited, p. 84–86.
TISDALE, W. H. RELATION OF TEMPERATURE TO THE GROWTH AND INFECTING POWER OF FUSARIUM LINI. *In* Phytopathology, v. 7, no. 5, p. 356–360, 1 fig., pl. 11. 1917.

Now it would be expected, and it is clearly true, that plants grown in soils held at such widely different temperatures would show in the given time considerable difference in size and degree of maturity. The first question which will be asked regarding this difference in nodule development will be whether it does not correspond more or less approximately with corresponding differences in root or shoot development. Does it show a trend distinctly different from that of other portions of the plant?

When the figures given in this table are presented in graphs, the differences in trend become obvious. A comparison of the weights of the nodules with those of the roots (fig. 1, 2) will show that in both

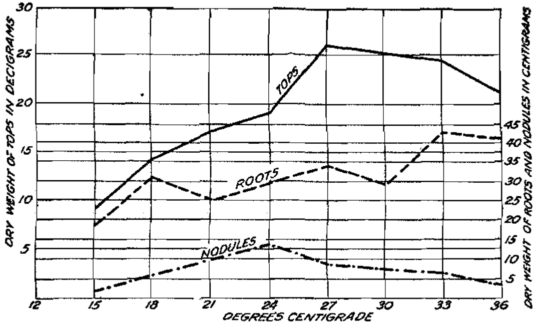



FIG. 2.—Comparison of dry weights of tops, roots, and nodules of soybeans given in Table III, grown during April and May.

series the maximum development of nodules occurs at 24° C., with very slight development at the extremes, 15° and 36°. Root development, on the other hand, rises much more rapidly at the lower temperatures and is maintained at the the higher temperatures, reaching a maximum in the second series at a point 9° higher than that of the nodules. Root development is far more uniform at all temperatures than is nodule development.

A comparison of nodule development with shoot development (fig. 1, 2) shows that the effect of temperature upon the development of the two structures is quite different. As with root development, shoot development is relatively more vigorous at 15° and 18° C. than is nodule development, which increases greatly at 21° and reaches a maximum at 24°. When at 27° the weight of nodules is beginning to diminish, that of shoots maintains its level or increases. Through the higher temperatures weight of nodules falls off rapidly, while that of shoots remains at the high level.

TABLE III.—*Dry weight per plant of shoot, roots, and nodules produced in 63 days in the first series, grown in November, December, and January, and 55 days in the second series, grown in April and May*

| Temperature. | First series. | | | Second series. | | |
|---|---|---|---|---|---|---|
| | Shoot. | Root. | Nodules. | Shoot. | Root. | Nodules. |
| °C. | Gm. | Gm. | Gm. | Gm. | Gm. | Gm. |
| 12 | 0. 133 | 0. 040 | 0. 000 | | | |
| 15 | .410 | .110 | .006 | 0. 922 | 0. 184 | 0. 021 |
| 18 | .432 | .135 | .008 | 1. 430 | .318 | .060 |
| 21 | .632 | .140 | .033 | 1. 710 | .269 | .108 |
| 24 | .875 | .131 | .043 | 1. 900 | .296 | .145 |
| 27 | .771 | .108 | .030 | 2. 620 | .342 | .094 |
| 30 | .818 | .096 | .014 | 2. 540 | .296 | .089 |
| 33 | .863 | .116 | .012 | 2. 440 | .437 | .076 |
| 36 | .996 | .108 | .005 | 2. 130 | .422 | .042 |

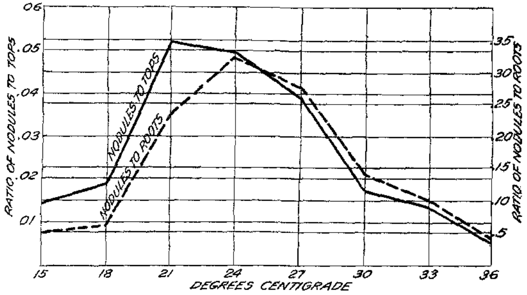



FIG. 3.—Ratios of weight of nodules to weight of tops and of roots. First experiment.

In order to obtain a clearer view of the contrast between the effect of temperature upon weight of nodules and that of roots and shoots, the ratios of the weights of these parts of the plant have been determined and plotted (fig. 3, 4). If there is a direct relation between development of nodules and that of either the aerial or subterranean parts of the plants—if nodule development is conditioned by top or root development quite independently of the temperature factor which was varied in these experiments—then the ratio should be approximately constant, or at any rate should be a straight line. A glance at the graphs shows that this is not the case. The ratios when plotted produce curves which are closely similar. No direct relation appears to exist between weight of nodules and that of either tops or roots under the conditions of these experiments. At 21° and 24° C. the weight of nodules is relatively larger than at temperatures above or below this region. The wide dif-

ference in the ratios and the consistent similarity of the curves can hardly be explained otherwise than as a temperature effect upon nodule development which is quite different from that upon development of root or shoot.

## EFFECT OF SOIL TEMPERATURE UPON THE COMPOSITION OF THE INOCULATED PLANTS

In order that comparisons might be made of the amount of nitrogen found in inoculated and uninoculated plants, total nitrogen determinations were made of shoots, roots, and nodules of the plants grown in each series. Since the uninoculated plants did not remain free from nodules, the desired comparisons can not be made. Nevertheless the

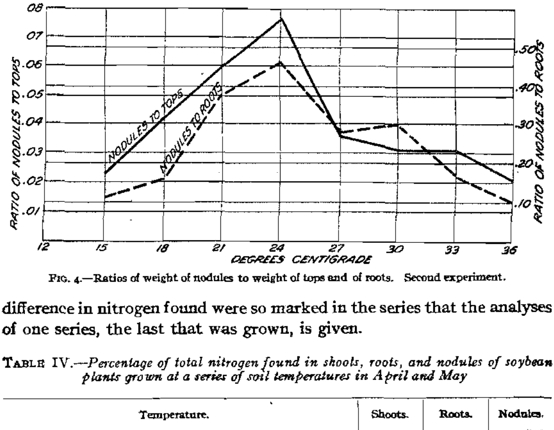



FIG. 4.—Ratios of weight of nodules to weight of tops and of roots. Second experiment.

difference in nitrogen found were so marked in the series that the analyses of one series, the last that was grown, is given.

TABLE IV.—*Percentage of total nitrogen found in shoots, roots, and nodules of soybean plants grown at a series of soil temperatures in April and May*

| Temperature. | Shoots. | Roots. | Nodules. |
|---|---|---|---|
| °C. | | | |
| 15 | 2.42 | 2.89 | 5.95 |
| 18 | 2.86 | 3.40 | 6.95 |
| 21 | 4.27 | 2.64 | 6.25 |
| 24 | 4.22 | 2.98 | 5.95 |
| 27 | 4.55 | 2.66 | 6.25 |
| 30 | 4.58 | 2.62 | 6.25 |
| 33 | 3.98 | 2.67 | 6.00 |
| 36 | 3.77 | 2.85 | 5.70 |

The greatest differences in content of nitrogen are found in the shoots, the roots being very uniform and the nodules hardly less so. It will be seen that, generally speaking, the high nitrogen content of the top is

correlated with the best development of nodules, though the curve which would be produced by these figures when plotted in the manner of the preceding data would not have the same shape. The largest amount of notrogen is found at a higher temperature than the point at which the largest dry weight of nodules was found. A rather sudden increase in nitrogen at 21° C. as compared with 18° and a sudden fall at 33° as compared with 30° has characterized the series obtained thus far. The result of the analyses which have been made seems worth recording; but whether the low nitrogen content of the plants grown at both ends of the series is wholly due to the small nodules found on these plants, and whether the high nitrogen content of plants in the center of the series is due to large and presumably efficient nodules, likely as this connection appears, remains to be determined by more refined methods.

## DISCUSSION OF FACTORS THAT MAY HAVE HAD AN INFLUENCE UPON THE DATA GIVEN

### CONCENTRATION OF NITRATES IN THE SOIL

In view of the fact already discussed, that large amounts of nitrates in the soil solution decrease nodule development and even inhibit it before the concentration becomes great enough to injure the plant directly, it is unfortunate that the soil used in these series should have had as much nitrate as was found (Table IV), even though the largest amounts are far below the inhibition point. There appears to be no data available in literature whereby we may know what is the maximum or the more usual amount of nodular structure formed on the roots of any of the legumes. Although the amount of nodular structure which peas may produce may be quite different from the amount which soybeans may produce under the most favorable conditions, yet it may be worth while to record here that the writers have found in one instance a variety of wrinkled peas producing at the blossoming stage nodules whose dry weight was 2.2 times as great as that of the entire root system (average of 25 plants); and in individual plants the ratio of weight of nodules to roots was as high as 4.5 to 1. However the ratio of weight of nodules to tops in these plants was 0.085 to 1, a ratio not much different from that found under the best experimental conditions for soybeans recorded here (fig. 3, 4).

However, the question of immediate interest here is whether or not the nitrate content of the soil used in these series was greatly changed at any of the temperatures at which it was held, and if there is any evidence that this change was of sufficient size and in the right direction to indicate that it may have been responsible for the increased or decreased nodule formation at this temperature. In order to obtain information regarding the change which soil temperature may have produced in the series, nitrate nitrogen determinations were made by

the colorometric method of a composite sample of the soil at the beginning of the experiment and of a sample from two cans of soil at each temperature when the plants were harvested. In addition, in order to get some clue to intervening changes, an unplanted can of soil was kept at each temperature from which a sample was taken at about the middle of the period. The results obtained are shown in Table IV. The effect of soil temperature does not appear to have been as definite and consistent upon the concentration of nitrate nitrogen as was anticipated. Until further data are available, it seems unwise to attempt to interpret the results. However, the very absence of large and consistent modification enables us to believe that this factor was not important in its effect upon nodule development. The only point at which nitrate accumulation became very large occurs in the second series where the unplanted soil shows at the end of 26 days a very high nitrate content at 21° and 24° C. If it is assumed that a similar concentration took place in the planted pot at an early stage in the development of the plants before they were large enough to reduce it by absorption, it would be anticipated that a reduction in nodule production would be found here. In fact, however, this point of high nitrate formation is the point of highest nodule production, just as it is in the first series where no evidence of high nitrate content at any time was obtained.

TABLE IV.—*Nitrate nitrogen in the soil in which the soybeans grew and also in unplanted soil*

| Temperature. | First series. | | | | Second series. | | | |
|---|---|---|---|---|---|---|---|---|
| | Planted soil. | | Unplanted soil. | | Planted soil. | | Unplanted soil. | |
| | At start. | At end. | After 24 days. | At end. | At start. | At end. | After 26 days. | After 61 days. |
| *°C.* | *P. p. m.* | *P. p. m.* | *P. p. m.* | *P. p. m.* | *P. p. m.* | *P. p. m.* | *P. p. m.* | *P. p. m.* |
| 12 | 20.5 | 8.6 | 16.3 | 14.4 | ........ | ........ | ........ | ........ |
| 15 | 20.5 | 3.3 | 11.4 | 8.3 | 90 | 20.0 | 34 | 34 |
| 18 | 20.5 | 8.6 | 16.5 | 10.6 | 90 | 14.0 | 56 | 34 |
| 21 | 20.5 | 8.1 | 19.3 | 20.5 | 90 | 18.0 | 124 | 15 |
| 24 | 20.5 | 10.6 | 11.8 | 14.4 | 90 | 8.5 | 150 | 17 |
| 27 | 20.5 | 10.6 | 13.9 | 9.2 | 90 | 14.0 | 88 | 30 |
| 30 | 20.5 | 16.9 | 13.9 | 8.3 | 90 | 4.8 | 36 | 58 |
| 33 | 20.5 | 3.3 | 15.1 | 3.6 | 90 | 4.8 | 29 | 50 |
| 36 | 20.5 | 9.7 | 21.8 | 3.6 | 90 | 30.0 | 22 | 30 |

## MOISTURE CONTENT OF THE SOIL

In view of the effect which high moisture content of the soil is reported by Wilson [1] and others to have in increasing nodule production, the moisture content of the soil in the later series was kept uniform at all

[1] WILSON, J. K. PHYSIOLOGICAL STUDIES OF BACILLUS RADICICOLA OF SOY BEAN (SOJA MAX PIPER) AND OF FACTORS INFLUENCING NODULE PRODUCTION. N. Y. Cornell Agr. Exp. Sta. Bul. 386, p. 363-413, fig. 80-94. 1917.

temperatures at one-half the moisture-holding capacity of the soil, previously determined to be 14 per cent of its dry weight. In order to get further evidence as to the extent of the effect of high moisture content, a single can containing three plants was placed at each temperature in the last series with moisture content of 18 per cent of the dry weight—a distinctly wet soil. Accidents which befell several plants in the series produce irregularities in the figures which would require long explanation. Suffice it to say here that though the tops were increased in size there is no evidence that the nodules were increased either in number or size. Apparently moderate differences in moisture content of the soil were not large factors influencing results in the previous experimental work where exact control of soil moisture was not accomplished.

### HYDROGEN-ION CONCENTRATION OF THE SOIL SOLUTION

Inasmuch as it was considered possible that the extreme temperatures at which the soil was held might produce changes which would alter the hydrogen-ion concentration of the soil solution, and hence the formation and perhaps development of nodules, a determination of this environmental factor was made toward the close of the last series described. Samples of soil were taken from the unplanted pots at 15°, 24°, and 36° C. A determination of the hydrogen-ion concentration of the soil solution of the three samples by the colorometric method gave identical results, the $P_H$ value being 6.3 in all three cases. Thus no evidence was obtained that temperature had altered this important factor in this series.

### SUMMARY

(1) Preliminary studies have been made upon the effect of soil temperature on the development of four legumes, alfalfa, red clover, field peas, and soybeans, with special reference to its effect upon the infection of these plants by *Bacillus radicicola* and the subsequent development of nodules. The larger part of the data were obtained by growing plants in soil held at a series of temperatures 3° apart from 12° to 36° C. The air temperature was uniform for all plants, ranging from 14° to 20°.

(2) As was anticipated, the four plants differed in their ability to tolerate soil temperatures at the ends of the series. Peas were dwarfed at 30° C., clover developed poorly at 36°, while alfalfa and soybeans still grew very well at 36°. Soybean plants grown in the soils held at 12°, 15°, 33°, and 36° showed very dark green color of leaves, whereas those toward the center of the series became progressively lighter, those at 24° being lightest.

(3) With regard to the number of nodules formed on plants grown in soil held at this series of temperatures, irregularities were found in each series; but no large consistent differences were discovered, except that at the extreme upper and lower limits at which a plant will survive the

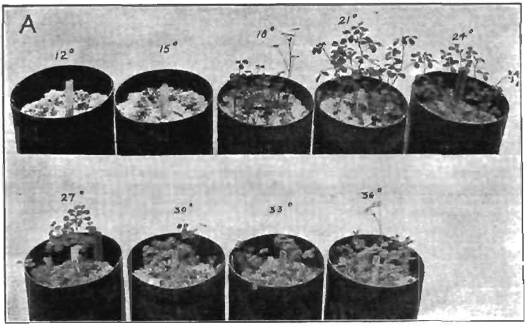
number is reduced, and that peas usually produced greatly increased numbers at 30° C. All these species form nodules in soils at any temperature at which the plant can make a growth that is at all vigorous.

(4) While variation in number was not consistent, size measured by the average dry weight per plant of all those formed on a number of plants was found to differ greatly and consistently within the series, at least so far as the soybean plant was concerned. The maximum weight attained on the soybean plant after a period of two months was found at a soil temperature of 24° C. Examination of nodules on the roots of the other legumes indicated that their maximum production occurred at about the same temperature.

(5) Weight of nodules produced by soybeans was not found to be correlated with the weight of tops or of roots through the series of temperatures. Weight of tops was almost or quite as great at 30° to 36° as at 24° C., while weight of nodules declined rapidly at the higher temperatures. Weight of roots likewise showed no such diminution at the higher temperatures or even at the lower temperatures as did weight of nodules. With the soybean plant, and to a much less marked degree with the other plants, there was a correlation between weight of nodules and color of plant, the largest weight of nodules occurring on plants with the palest green color.

(6) Generally speaking, plants with large nodules had a higher percentage of total nitrogen in the tops, though this correlation is not exact.

(7) Factors of soil environment that are regarded as having an influence upon nodule formation have been taken into account. Soil moisture has been controlled within narrow limits. Concentration of nitrates and the hydrogen-ion concentration of the soil solution have been recorded. It is not believed that variations in any of these factors are to be regarded as having produced the variations in nodule development recorded at the different temperatures in these series.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18°
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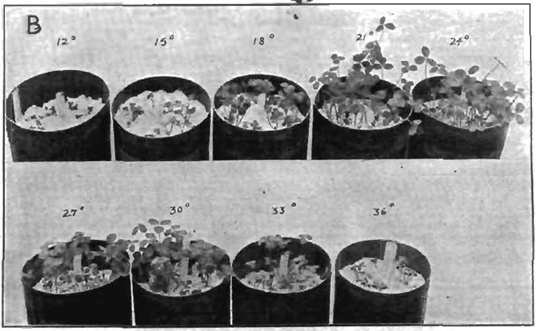
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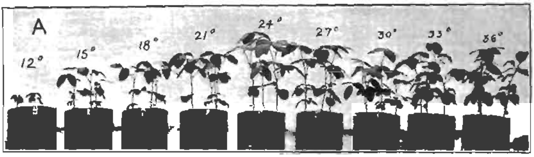
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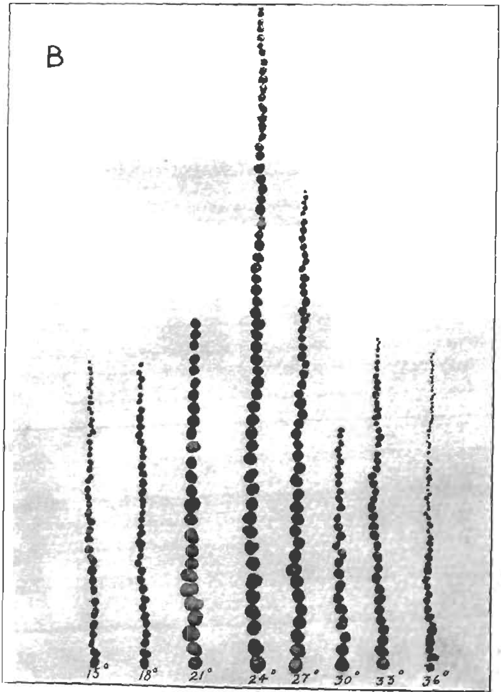
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15°
18°
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PLATE 2

A.—Soybean plants grown 63 days in soil held at the temperatures indicated.

B.—Nodules from 6 soybean plants (only 5 plants at 30° C.) grown 63 days at the temperatures indicated. One-half of the plants are shown in A.

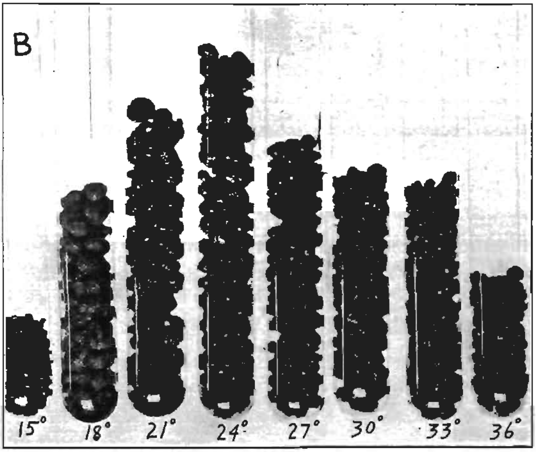
54817°—21——3

PLATE 3

A.—Soybean plants inoculated with *Bacillus radicicola* contrasted with uninoculated plants grown 55 days in soil held approximately at the temperatures indicated. The pots are grouped according to temperature, with the control on the left and the inoculated pot on the right in each set.

B.—Nodules from 9 soybean plants grown 55 days in soil held at approximately the temperatures indicated.

A
15°
24°
36°


B
15°
18°
21°
24°
27°
30°
33°
36°

# INFLUENCE OF THE PERIOD OF TRANSPLANTING WESTERN WHITE PINE SEEDLINGS UPON THEIR BEHAVIOR IN NURSERY AND PLANTATION

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At forest nurseries in the northern part of the United States the work is customarily crowded into three or four weeks in spring immediately following the time when the soil can first be worked. Preferably, the stock is lifted, packed, shipped early, and spring sowing and transplanting are all usually crowded into this period. At the Savenac Nursery, Haugan, Mont., this spring congestion has been keenly felt; and the experiments outlined below have had for an object the determination of the safe limits of the transplanting season. The results may or may not apply beyond the local conditions prevailing in the region of western Montana and northern Idaho.

## FALL TRANSPLANTING

Work was commenced upon this problem at the Savenac Nursery in the fall of 1913. By the use of the Mast trencher method, 600 1-year-old seedlings of western white pine (*Pinus monticola* Dougl.) were transplanted on each of the four following dates: August 15, September 1, September 15, and October 10. In May, 1914, these plants were examined and the overwinter losses were recorded. Loss by frost heaving, as indicated in the figures, includes not only plants completely thrown out but also those lying prostrate on the ground, even though they were quite firmly attached and still alive, because in that condition they would never recover sufficiently to be fit to plant. A few individuals showed the symptoms of winterkilling. These were about evenly distributed among the four units, in no case amounting to 1 per cent of the total. Figure 1 shows the loss by frost heaving. From one-third to one-half of the plants were heaved out during the cold nights of late October, before the coming of snow, and during the clear weather of late March and early April after the snow left. The loss was greatest in the October 10 unit. This may be explained by supposing that, because of the warmer soil temperature the individuals transplanted earlier had had time to make sufficient root growth to render them more resistant to the frost lifting, but that those transplanted latest were virtually heeled in. However, precise evidence on this point is lacking.

As it had been suggested that possibly in the Mast V-shaped trench a pocket of loose soil was formed around the lower roots and that this

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predisposed the plants to heaving, several rows of 1–0 western white pine seedlings, transplanted September 25, 1913, in open plowed trenches were likewise examined in May, 1914. From a total of about 12,000 trees the loss from frost heaving was 29.6 per cent and that from winter-killing 1.2 per cent. Here, again, nearly one-third of the plants were thrown out—a loss hardly 6 per cent less than that by the Mast trencher

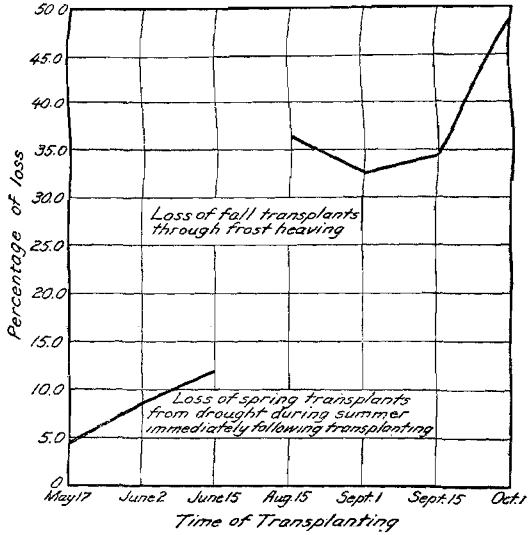


FIG. 1.—Loss by frost heaving of seedlings transplanted at different dates.

method, which was used 10 days earlier. Apparently the method mattered little, the loss having been a necessary consequence of fall transplanting.

Lorey [1] found that fall transplants of Douglas fir and European larch led spring transplants in height growth in the transplant rows. More recently, Toumey [2] states that fall transplants lead in earliness of growth and in size, provided they escape winter injury. While no data with

[1] LOREY, Tuisko. MITTHEILUNGEN AUS DEM FORSTGARTEN UND KULTURBETRIEB. II. FORSTGARTEN INSBESONDERE. *In* Allg. Forst u. Jagd. Ztg., N. F., Jahrg. 70, p. 193–197. 1894.

[2] TOUMEY, James W. SEEDING AND PLANTING: A MANUAL FOR THE GUIDANCE OF FORESTRY STUDENTS . . . xxxvi, 455 p., 140 fig. New York, 1916.

respect to those matters were collected, the heavy loss through heaving at Savenac Nursery more than balanced any possible gains of that kind. Fall transplanting is clearly so unsafe that no further local experiments with it are necessary.

## SPRING TRANSPLANTING

### NURSERY TESTS

Experiments in the spring of 1913 had for their object the comparison of three lots of 600 1–0 western white pine each, transplanted on May 17, June 2, and June 16. The first summer's loss from drought increased with the lateness of the transplanting period, as is shown also by figure 1. The June 16 lot looked less thrifty than the others at the end of the season, and it was concluded that[1] in case of necessity, transplanting could evidently be continued as late as June 15, though it is not desirable.

As a control on the tests made in the spring of 1913 it was arranged to transplant 1,000 1–0 western white pine at Savenac Nursery every 10 days during the spring of 1914. This was actually done on April 24, May 1, May 9, May 20, May 30, June 12, June 19, June 30, and July 14. On each of these dates 100 more were removed from the seed-bed, of which the weights and measurements appear in Table I.

TABLE I.—*Weights and measurements of 1–0 western white pine on different dates of transplanting in the spring of 1914*

| Date of transplanting. | Lot No. | Average length of stem. | Average diameter of stem. | Average weight. | | Percentage of plants with buds closed. | Percentage of plants with buds swelling. | Percentage of plants with buds open. | Average new spring growth. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Top. | Root. | | | | Needles. | Rootlets. |
| | | *Inches.* | *Mm.* | *Gm.* | *Gm.* | | | | *Inches.* | *Inches.* |
| Apr. 24 | 1 | 1.54 | 1.17 | 0.140 | 0.115 | 100 | 0 | 0 | 0.0 | 0.05 |
| May 1 | 2 | 1.52 | 1.23 | .153 | .122 | 100 | 0 | 0 | .0 | .10 |
| 9 | 3 | 1.50 | 1.13 | .143 | .090 | 90 | 10 | 0 | .0 | .20 |
| 20 | 4 | 1.90 | 1.19 | .186 | .120 | 0 | 34 | 66 | .08 | .54 |
| 30 | 5 | 1.99 | 1.17 | .125 | .081 | 1 | 6 | 93 | .15 | .62 |
| June 12 | 6 | 2.30 | 1.28 | .265 | .150 | 0 | 1 | 99 | .27 | 1.07 |
| 19 | 7 | 2.43 | 1.22 | .203 | .103 | 0 | 0 | 100 | .44 | .94 |
| 30 | 8 | 2.30 | 1.46 | .313 | .139 | 0 | 0 | 100 | .73 | (1) |
| July 14 | 9 | 2.60 | 1.53 | .274 | .130 | 0 | 0 | 100 | 1.02 | (1) |

1 Not recorded.

In Table I stem length is the distance from the ground line to the tip of the terminal growing point. The stem diameter was measured at the ground line. The average new growth of rootlets was based upon the longest rootlet noted in each plant examined and not upon all new rootlets. This figure is only relatively correct, because some slight root breakage was unavoidable in taking up the plants from the seed bed. Root growth data were omitted in the two latest lots, since the older portions of the

1 Unpublished progress report.

new roots were assuming a brown, mature appearance, and this made it difficult to determine the margin of growth.

Figure 2 shows graphically the condition of the seedlings as to length of stem and spring growth of needles and rootlets on the different dates of transplanting.

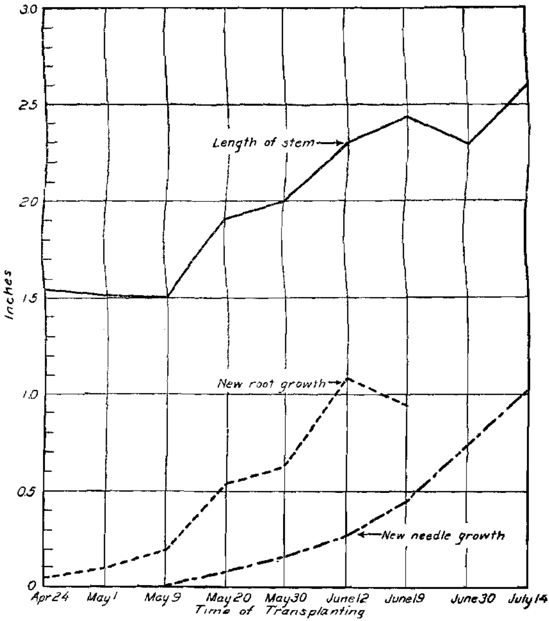


FIG. 2.—Length of stem and spring growth of needles and rootlets of seedlings transplanted at different dates.

Table I brings out the following points:

1. Root growth began prior to April 24 during the spring of 1914, or over three weeks before visible stem growth. Although not so indicated by the table, it was found that the earliest visible root elongation took place in the superficial soil layers, gradually progressing to deeper and deeper levels as the season advanced, presumably in response to changes in soil temperature.

2. The swelling of the buds began a little before May 9, and two-thirds of them were fully open by May 20.

3. Although individual variations existed—due largely, it is thought, to a lack of uniform density in the seed bed—stem length, stem diameter, and weight of top increased in general as the season advanced.

4. The proportion of the fresh weight of the plants contained in the root was greatest early in spring and decreased as the growth pushed ahead in May and June. Figure 3 brings out this point. Owing to the possibility of variation in the water content of top and root, dry weights would be of interest, but circumstances prevented their being obtained.

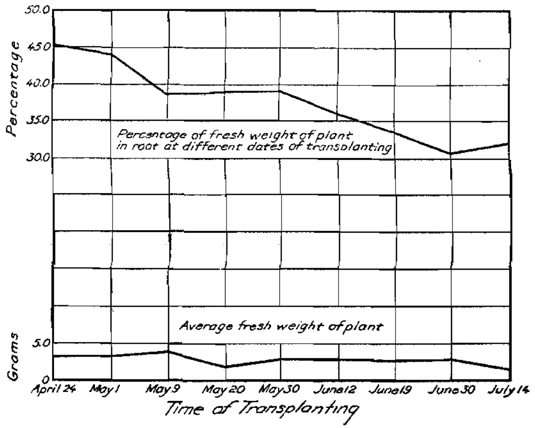


FIG. 3.—Proportion of fresh weight of roots of seedlings transplanted at different dates.

Transplanting was done in adjacent parallel rows, and these were irrigated at intervals during the summer of 1914. As early as August 1 there had come to be a marked differentiation in size and color. The April 24, May 1, and May 9 units showed particular vigor and had a rich green color. The June 30 and July 14 lots showed much the same development as the earliest ones but had a yellow color, which gave the July 14 lot an almost sickly appearance. The remaining intermediate lots showed a healthier color than the later ones but lacked the size and development of the latter. This differentiation was increasingly marked at the end of the growing season. The loss from drought in these lots during the summer was very slight, the heaviest loss, 2.4 per cent, being suffered by the July 14 unit.

On September 11, 1914, the season's growth of stem and needles was obtained by measuring every tenth plant in each lot, or 100 in each unit. The terminal buds of 500 plants in each lot were examined as to their maturity on this same date. Buds having a definite form, of a deep brown color, and covered with a protective coat of fine hairs were classed as mature. Plants without a single well-defined bud and those whose growing point had a tender green color, without the coat of hairs, were considered of immature development. For purposes of comparison, data similar to the foregoing were obtained from 2-year-old western white pine plants from a representative area of seed bed. These plants were of the same age and seed lot as the nine transplanted units, differing only in that they had been allowed to remain in the seed bed. These data are given in Table II.

TABLE II.—*Growth and development of western white pine during the first season in the transplant bed*

| Date of transplanting. | Lot No. | Average seasonal stem growth. | Average seasonal growth of needles. | Percentage of plants with mature buds. | Percentage of plants with immature buds. |
|---|---|---|---|---|---|
| | | *Inches.* | *Inches.* | | |
| Apr. 24 | 1 | 0.757 | 0.934 | 80.0 | 20.0 |
| May 1 | 2 | .729 | .952 | 75.7 | 24.3 |
| 9 | 3 | .763 | .897 | 75.1 | 24.9 |
| 20 | 4 | .734 | .424 | 69.1 | 30.3 |
| 30 | 5 | .825 | .566 | 70.1 | 29.9 |
| June 12 | 6 | .874 | .570 | 69.6 | 32.4 |
| 19 | 7 | .918 | .611 | 56.4 | 43.6 |
| 30 | 8 | .974 | .715 | 80.6 | 19.4 |
| July 14 | 9 | .951 | .943 | 92.6 | 7.4 |
| Not transplanted | ...... | 1.063 | 1.131 | 93.5 | 6.5 |

By average growth of stem and needle is meant the growth for the entire season, regardless of whether that growth took place in the seed bed, in the transplant bed, or in both. Needle measurement was made in the middle of the sector of currently-grown stem.

Table II brings out the following points:

1. The later the transplanting after the buds open, the higher the stem growth for the season. It appears that height growth practically ceases for a time after transplanting, the plant's energies being directed toward getting established in its new habitat. In other words, the height growth is roughly proportional to the length of time the plant is left in the seed bed. Hence, plants that were not transplanted made a higher stem growth than any of the transplanted lots.

2. The longest needle growth at the end of the season had been made by the first and last lots (Apr. 24 and July 14). The needle growth of the first lot had been made entirely in the transplant bed and was accompanied by a deep green color, but that of the last lot had taken place in

the seed bed before transplanting and the needles of these looked much less vigorous. As figure 4 shows, the season's needle growth commences to fall with the May 9 lot, drops abruptly with the May 20 lot, then climbs gradually until the last lot equals the earliest. The stock that was not transplanted produced longer needles than any of the transplanted units.

3. The difference in dates of transplanting had a pronounced effect upon the maturing of the fall buds. The earliest maturing lots were the two that were transplanted latest (June 30 and July 14). The less

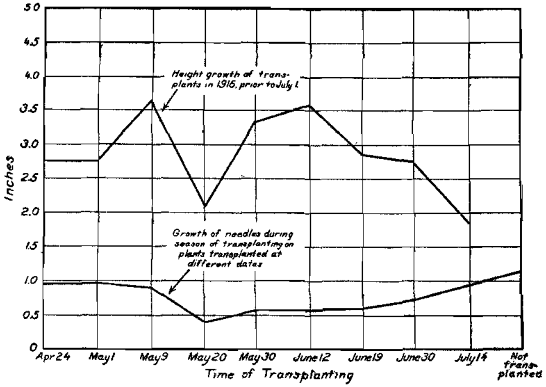


FIG. 4.—Increase in height and growth of needles of seedlings transplanted at different dates.

favorable weather conditions appear to hasten preparations for winter by stock transplanted in summer.

4. Seedling stock of the same age and source (2–0) led transplants (1–1) in current stem growth and needle development no matter what the period of transplanting. The shock of the treatment, expressed quantitatively, resulted in a loss of 0.3 inch of stem growth and 0.2 inch of needle growth, even when the transplanting was done at the most favorable period.

At the end of the season in which the transplanting was done it appeared that the plants lined out before the buds were open had suffered the least shock, and, judging from their unhealthy appearance, those transplanted in midsummer (July 14) seemed to have suffered most. No single item of weight or measurement appears to be a consistent indicator of the degree of severity of the shock.

On July 1, 1916, 100 plants were washed out from each of these nine transplanted units by the aid of water under pressure. At this time, the stock could be considered to be in the 1–2½ age class. Data from these 900 plants are assembled in Table III.

TABLE III.—*Weights and measurements of 1–2½ western white pine*

| Date of trans-planting. | Lot No. | Average stem height growth (current).[a] | Average stem diameter. | Average number of laterals. | | | | Average total fresh weight of plant. | Percentage of weight in root. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | First order. | | Second order. | | | |
| | | | | 2 inches and up. | 0.5-inch to 2 inches. | 2 inches and up. | 0.5-inch to 2 inches. | | |
| | | *Inches.* | *Mm.* | | | | | *Gm.* | |
| Apr. 24 | 1 | 2.75 | 6.31 | 10.2 | 5.4 | 3.4 | 15.3 | 3.09 | 43.4 |
| May 1 | 2 | 2.75 | 6.72 | 10.3 | 6.7 | 6.3 | 27.4 | 3.16 | 45.6 |
| 9 | 3 | 3.66 | 7.14 | 10.3 | 6.1 | 4.1 | 17.2 | 3.78 | 36.2 |
| 20 | 4 | 2.09 | 5.65 | 9.0 | 6.1 | 3.2 | 13.0 | 1.84 | 45.7 |
| 30 | 5 | 3.27 | 6.48 | 10.5 | 4.5 | 2.9 | 13.0 | 2.78 | 41.4 |
| June 12 | 6 | 3.58 | 6.48 | 8.1 | 5.2 | 3.6 | 11.3 | 2.74 | 36.9 |
| 19 | 7 | 2.85 | 6.07 | 9.1 | 5.3 | 3.7 | 10.5 | 2.54 | 39.4 |
| 30 | 8 | 2.74 | 6.27 | 10.2 | 5.1 | 3.8 | 12.4 | 2.62 | 42.7 |
| July 14 | 9 | 1.82 | 4.95 | 2.6 | 5.3 | 2.5 | 9.9 | 1.41 | 48.3 |

[a] By current stem growth is meant the 1916 growth prior to July 1.

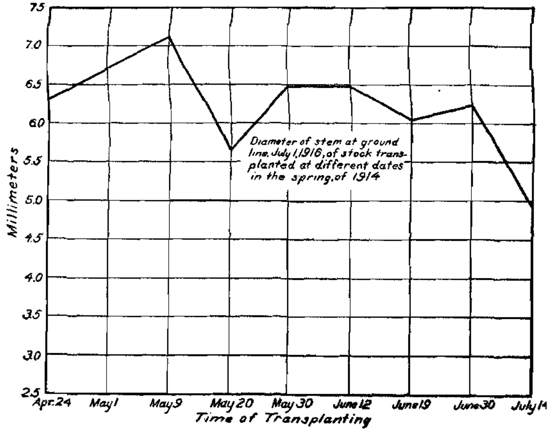


FIG. 5.—Increase in diameter of stem of seedlings transplanted at different dates.

A study of the proportion of the fresh weight of plant in the root system shows that the relation between this proportion and the time of transplanting, which was so evident two years before, had entirely disappeared. · All the plants were washed off, and the surfaces were allowed to dry in the air; but as the evaporating power of the air varied, it was not possible to compare directly the average fresh weights obtained at the time of transplanting with those obtained on July 1, 1916. All the weights obtained on the latter date are, however, comparable with one another. Figures 3, 4, and 5 illustrate further the current height growth of stem, the stem diameters, and the total fresh weight of the plants.

A striking similarity will be noted in the curves in figures 3 (weight), 4, and 5. All agree in showing two minima, the first for the May 20 lot, and the second and still lower point for the July 14 transplanting. This bears out the conclusion reached in the fall of 1914 that the shock of transplanting fell hardest upon the July 14 lot. But it now appears that the transplanting on May 20 was almost equally lasting in its unfavorable influence upon growth in the transplant bed. Between these two dates there appears to have been a period of about a month in which transplanting worked less injury to the plant.

As the roots of these plants were removed by washing, and with a minimum of breakage, it seemed worth while to compare the average numbers of laterals of the first and second orders. Figure 6 shows graphically the data obtained. In order to bring the curves close together, and thus facilitate comparison, the actual average number of lateral rootlets of the second order, between 0.5 and 2 inches in length, has in each case been divided by 2 in plotting the curves, and the average total number of lateral rootlets in the four classes has similarly been divided by 5. Because of the difficulty involved in recording them, and the limited time available, no records were obtained of the number of laterals of higher orders than the second, nor, in any case, of laterals less than 0.5 inch in length. The figures obtained are, however, considered indicative of the general nature of the root system.

There is a close relation not only between the average number of laterals in each of the two length classes of the second order but also between these and the total number of rootlets of the recorded classes. On the other hand, the curves for the two classes of the first order run quite differently. Yet, so far as determining the total curve is concerned, these two classes could obviously have been neglected. There is a consistent decline in the fibrous development of the root system as the transplanting season advances. Transplanting in early summer, and even more so in late summer, holds back lateral root growth in the transplant bed, the effect being strikingly noticeable two years afterwards.

Although several seasons' observations had shown no noticeable loss through winter frost heaving of 1–0 western white pine transplanted in April and in early May, it was found in the spring of 1918 that of 2,922 1–1 white pine transplanted June 15, 1917, in connection with another experiment, 1,152, or 39.4 per cent, were heaved out during the late fall, winter, and early spring of 1917–18. This is nearly as heavy a loss

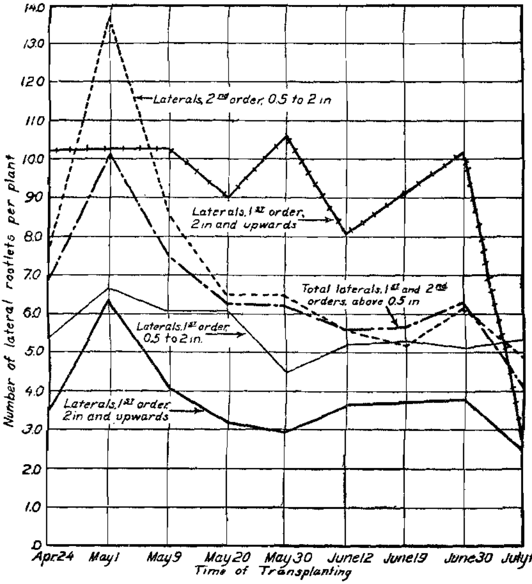


FIG. 6.—Number of lateral rootlets on seedlings transplanted at different dates.

as had previously been recorded for fall transplanting. However, an unprecedented snowless period in December probably contributed to produce this result. A greater susceptibility to frost heaving on the part of late spring and summer transplants is the natural result of the poorer root development just referred to. The plant must rebuild its entire root system late in the season and so has a relatively poor anchorage when the frost comes. Furthermore, Cannon [1] has found that the

[1] CANNON, William Austin. ROOT HABITS OF DESERT PLANTS. 96 p., 17 fig., 23 pl. Washington, D. C., 1911. (Carnegie Inst. Wash. Pub. no. 131.)

formation of an abundant lateral root requires a favorable water content in the soil and a sufficiently high soil temperature. Although summer soils are warm, yet, in spite of occasional irrigation, the greatest loss of transplants from drought at Savenac Nursery occurs during July and August, indicating that there is less available soil moisture during that period, or at least that there is a smaller balance for growth when the transpiration loss of the plant has been met.

It seems, therefore, that in the foregoing series the plant organism was most deeply disturbed by being transplanted in midsummer. This appeared to be a consequence of the high evaporation and lack of moisture in the soil, along with the greater topheaviness of the plant. The May 20 transplants gave evidence of having been most severely set back, a result which must be attributed either to external conditions or to the internal state of the plant. The Savenac Nursery weather records show a precipitation of 2.16 inches in April, 1914, well distributed throughout the month, with only seven clear days. In May, previous to the twentieth, there fell 0.58 inch of rain, and 12 out of 19 days were cloudy or partly cloudy. On May 20, the soil was well stored with water and was favorable for the reception of plants. The maximum temperature on that day was 72° F., and it and the eight days following were partly cloudy. During the period from May 22 to 28, inclusive, 0.46 inch of rain fell, every day yielding at least a trace. The weather and soil conditions were, therefore, sufficiently favorable to convince the writer that the reason for the marked checking of the growth of the May 20 lot lay in the developmental stage of the plant itself. One-year-old western white pine seedlings, whose buds are just opening and whose tiny new needle fascicles are less than 1/10 inch long, show a particular sensitiveness toward removal and replanting.

## FIELD TESTS

The influence of the season of transplanting upon the behavior of the tree in the plantation is of special interest to the forester. One hundred of the plants from each of the nine spring lots described above were planted October 6, 1915, on the Wallace experimental area, near Wallace, Idaho. A northwest aspect—a typical white pine planting site—was selected. One row was devoted to each lot, and the rows were placed adjacent to each other and parallel, extending up and down the slope. The place where each tree was to be planted was previously marked by a cedar stake 16 inches long, whose top had been dipped in white paint to make it conspicuous among native cover plants. Each stake bore its lot number in black lumber crayon. The same man planted all the rows, using a uniform method.

On September 21, 1916, and on November 7, 1917, these plants were examined, their condition was noted, and the average height growth of stem was recorded, this average being based upon all vigorous living plants. Table IV gives the principal data secured.

TABLE IV.—*Date of transplanting, average current height growth, and percentage of trees surviving at the end of the 1916 and 1917 field seasons on the Wallace area*

| Date of transplanting—1914. | Lot No. | Average current height growth. | | Percentage of trees surviving. | |
|---|---|---|---|---|---|
| | | 1916 | 1917 | Fall 1916. | Fall 1917. |
| | | *Inches.* | *Inches.* | | |
| Apr. 24 | 1 | 0.57 | 0.62 | 92.2 | 90.9 |
| May 1 | 2 | .91 | .83 | 95.7 | 95.7 |
| 9 | 3 | .95 | .96 | 94.7 | 93.6 |
| 20 | 4 | .82 | .81 | 94.6 | 94.6 |
| 30 | 5 | .67 | .75 | 96.7 | 92.5 |
| June 12 | 6 | .96 | .95 | 96.8 | 92.8 |
| 19 | 7 | .82 | .84 | 96.8 | 93.7 |
| 30 | 8 | 1.00 | 1.15 | 98.9 | 98.9 |
| July 14 | 9 | .65 | .68 | 96.6 | 96.2 |
| Average of all lots | ...... | .82 | .84 | 95.9 | 94.3 |

The fact that the unusually dry summer of 1917 caused almost negligible losses makes improbable any further changes of importance in the survival standing of the nine lots. The percentage of living trees of all lots in the fall of 1917 was above 90. There is no superiority on the part of the early lots, the April 24 units standing lowest. The later lots have, on the whole, lived best. Both the May 20 and July 14 plants, while outclassed in the transplant bed, showed better than an average survival in November, 1917. In fact, the time of transplanting had no apparent influence in the field.

Figure 7 further illustrates the height growth of these plants. There is a marked similarity between the growth curves for 1916 and 1917; hence each resembles the total growth curve for the entire two seasons. For instance, in each of the three curves the average point for the June 19 lot falls exactly upon the horizontal average line for that curve. To facilitate comparisons, the height growth curve from figure 4 is plotted in figure 7 also. This renders it possible to compare the growth made in the plantation the first year after planting (1916) with that made the same season prior to July 1 by individuals left in the transplant beds (curves A and D, respectively).

There are certain points of resemblance between the curves of growth in transplant bed and in field. The May 1 and June 19 lots stand upon or very near the horizontal average line in both. The July 14 transplants stand low, and the May 9 and June 12 transplants stand high in both. But, on the other hand, the May 20 lot, which had a low growth rate in the

transplant bed, has an average rate in the field; but the May 30 and June 20 units reverse their positions with respect to the horizontal average lines. The planting out of the stock has evidently caused a somewhat

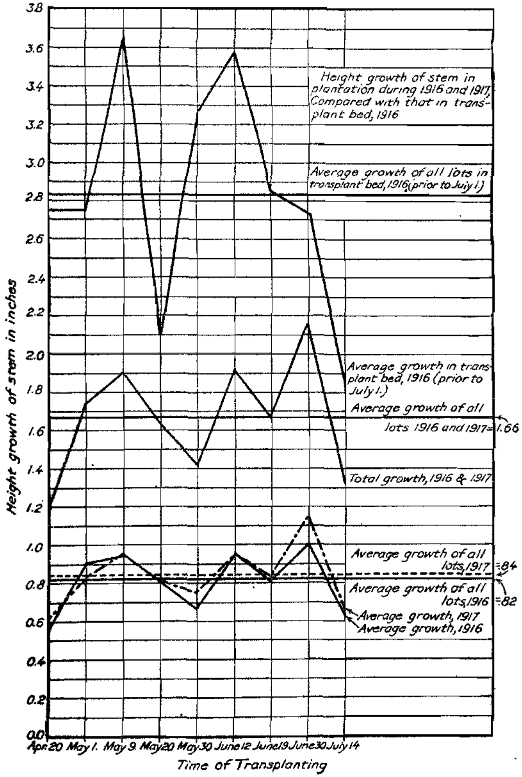


FIG. 7.—Increase in height of seedlings transplanted at different dates.

general rearrangement of growth rates in which the later lots tend to overtake the earlier ones, this rearrangement being accentuated after a second season in the field. The inferiority of the May 20 plants has

disappeared; the July 14 stock slightly surpasses the April 24 lot; and there is nothing to indicate that summer transplants are not fully the equal of spring transplants, so far as growth after planting is concerned.

## CONCLUSIONS

Transplanting western white pine seedlings at any time in the fall is not a safe practice at Savenac Nursery, because the frosts of the following late fall and early spring heave out the young plants.

Results in the plantation thus far indicate that, where 1–2 stock is grown, the transplanting season may be extended from a date as early in spring as the ground can be worked until early July. The shock of removal from the seed bed is greatest when the transplanting is done in midsummer, on account of the high evaporating power of the air, the low water content of the soil, and the greater top-heaviness of the plant with its considerably increased transpiring surface. A particularly severe shock was also suffered when transplanting was done at the time of bud opening and before the rudimentary needle fascicles had reached $\frac{1}{16}$ inch in length. Stock transplanted at either of these critical periods lagged behind the other lots for at least two years in the transplant bed. However, when they were planted in the field as 1–2 stock, the plants survived as well as the others, with little, if any, inferiority in growth on account of the considerable rearrangement of growth rates following the planting.

There are, nevertheless, other reasons which make transplanting at Savenac Nursery safer in spring than in summer. June and July transplants suffer more from drought the first season and, because of their poor root development at the end of the growing season, are more subject to frost heaving the following winter. The first disadvantage can be met by proper irrigation, but the second can not readily be prevented.

The safest practice, therefore, is to confine this work as much as possible to April and early May.

The foregoing conclusions apply to 1-year-old seedlings which are to remain two years in the transplant rows, 1–2 stock being the only age class of white pine transplants at present grown at Savenac Nursery.

Certain points brought out by this study may have an important bearing upon the season for field planting. It is probable, for instance, that subsequent lateral root development in the plantation may be decisively influenced by the time of planting.

# A DRYROT CANKER OF SUGAR BEETS

By B. L. Richards

*Department of Plant Pathology, Utah Agricultural Experiment Station*

What appears to be an undescribed rootrot of the sugar beet was first called to my attention on August 5, 1920, by Mr. A. H. Bateman. Specimens of the diseased beets collected at this date at Cornish, Utah, exhibited numerous brown, circular lesions that varied from $\frac{1}{16}$ inch to 1 inch in diameter (Pl. 4; 8, D; 9, A, B). The outer surface of the root covering these lesions, which in most cases remained entire, had so sunken as to give a definite undulating contour of alternating light and dark brown concentric areas or rings (Pl. 4). The removal of this outer layer of cells of the older lesions exposed deep cankers or pockets filled with hyaline-mycelium embedded in the dry remains of partially decayed host cells. This accompanying mycelium, when exposed to the atmosphere through the cracking open of the outer covering, appeared dark brown in color and immediately suggested the typical mycelium of the sterile, or "Rhizoctonia," stage of *Corticium vagum* B. and C. The general prevalence of black sclerotial bodies on the outside of the diseased beets (Pl. 7, A), together with the microscopic examinations made at this time, confirmed this initial suggestion.

An examination of the field from which these first diseased specimens were taken revealed the trouble to be of considerable economic importance; at least 20 per cent of the beets in this field of 40 acres were diseased. The disease appeared to be confined to definite areas wherein every beet might be found infected. These diseased spots varied considerably in size and appeared to be widening most rapidly in the direction parallel with rows. Three adjacent fields were found at this time to be seriously diseased, but none to the same degree as the field first visited.

The progress of the disease in these fields appeared of such ominous character as to require immediate investigation. However, as the season was well advanced, little more than preliminary experiments were undertaken. The results to date, while definite, are not sufficiently extensive to warrant final conclusions, and many of the important relations of the disease remain obscure; nevertheless it is felt that the apparent economic importance of the trouble justifies a preliminary description at this time.

The disease is first detected in the field by abnormal wilting of the leaves in the daytime with partial or complete recovery at night. Later

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the older leaves fail to recover, turn brown, and die. This dying of the outer or older leaves continues with the progress of the disease in the root until all the leaves on the affected beets may succumb. Localized browning frequently occurs in the blade and petiole, but to date no suggestion of a parasitic relation has been found. Neither the petiole decay reported by Duggar (*2*)[1] nor the "western crownrot" described by Edson (*3*) have been found associated with the dryrot canker in the field. A peculiar type of crownrot, however, is found late in the season, usually well toward harvest time (Pl. 6; 8, B). A study of a number of these crownrot specimens indicates definitely that the causal organism enters the beet below the surface of the soil and works upward in the tissues, eventually destroying the crown. The fungus has not been observed to attack the beet above the soil line.

It is evident that the fungus is unable to destroy the outer corky cells of the beet root, but gains entrance to the inner tissue at a definite point and works tangentially just beneath this outer layer. As the fungus eats its way from the point of entrance the outer tissues, due to killing and subsequent drying out of the cells beneath, sink in such a manner as to produce the circular lesion with its very definite undulating contour of alternating raised and sunken concentric "rings" (Pl. 4). The lesions appear first as a small, brown, sunken spot with a minute perforation in the center (Pl. 8, D). The first definite concentric "ring" which is considerably sunken below the central area and usually dark brown in color is noted before the lesion reaches a diameter of ⅛ inch. With continued enlargement a second and somewhat broader "ring," less sunken and much lighter in color, results. Similar concentric areas are developed alternately until the fungus reaches its limit of lateral spread. Individual lesions resulting from a single point of infection may obtain a size of from ¾ to 1 inch in diameter and develop as many as eight alternate "rings" (Pl. 4). When, however, adjacent lesions become confluent, as they frequently do (Pl. 4; 8, D; larger lesions result which may in severe cases cover a large part of the root surface. In such cases large concentric rings are produced, which become common to a number of centers of original infection (Pl. 4; 8, D; 9, A, B).

Another characteristic feature of the disease results in cases where infection occurs at or near the apex of the root. The root in such an event is usually severed at the point of infection and the fungus advances upward, producing the typical dryrot with resultant concentric rings which may encircle the entire root (Pl. 7, B). Again, cankers may occur with such frequency as to girdle completely the root (Pl. 8, A).

The distinctive feature of the contour, as shown in Plates 4 and 8, D, is obtained usually before the fungus penetrates deeply into the tissue of the beet and before a serious rupture of the outer layer occurs. With

[1] Reference is made by number (italic) to "Literature cited," p. 52.

the drying out and final cracking of this outer covering the fungus, possibly because of a better oxygen relation, eats radially into the beet, producing deep cankers (Pl. 5 and 6). The decaying tissues rapidly dry out as the fungus advances inward, leaving the cavity partially filled with a dry, pithy residue. Frequently the content of the canker appears as a definite plug, which, upon wetting, may be removed intact from the cavity of the canker (Pl. 9, D).

Except for slight cracking, the outer layer of dead cells remains entire and furnishes a definite covering until the lesion has reached approximately its limit of tangential spread. As the cells of this outer covering finally dry out the central perforation enlarges and ultimately gives rise to a definite crack which may extend the entire diameter of the lesion (Pl. 4; 8, D; 5). Frequently adjacent cracks become confluent, resulting in large characteristic fissures, which in severe cases of the disease may obtain from 2½ to 3 inches in length and from ½ to 1½ inches in depth (Pl. 5, 6). With numerous points of attack the beet by harvest time is converted into a dry, brittle shell filled with a pithy mass of host and fungous débris (Pl. 6).

During the season careful study was made of a large number of the beets taken from each of the different fields in which the dryrot had been found. In all cases the characteristic cankers exhibited the presence of the sterile stage of *Corticium vagum*. This fungus, it was found, may be obtained regularly in a pure form from any part of the typical canker, provided the outer covering of the lesion is not previously destroyed. The brown layer separating the normal from the diseased tissue (Pl. 8, A–C) has never failed to yield the fungus free from other organisms, and even from the open lesions cultures have been obtained with remarkable ease and regularity. The degree to which other organisms are found to be excluded is phenomenal.

To determine the etiological relation of the fungus, inoculations were made September 3 on partially grown beets. In the process of inoculation the soil was removed to a depth of approximately 4 inches from 21 beets in each of five rows. Each of the 21 beets in the first row was punctured a number of times with a sterile needle, and the inoculum, consisting of the beet fungus, grown for several days on potato agar, was then scattered throughout the soil as the latter was replaced about the beet. Row 2 was inoculated exactly as row 1 except that in place of needle punctures slight incisions were made in the beet by the use of a sterile scalpel. The beets in rows 3 and 4 were wounded as in row 1, and the soil was inoculated with two different "strains" of *Corticium vagum*.[1] Row 5 was left uninoculated, and the beets after wounding as in rows 1 and 2 were covered and grown as controls. All the wounded

[1] These "strains" were obtained from the surface of a potato tuber in 1918 and have proved virulent on potato stems in both sterilized and unsterilized soil.

beets in the control row healed normally. Infection occurred on but one beet in rows 3 and 4. The other beets in these two rows healed as perfectly as in row 5. The results of inoculation with the sugar-beet strain of the fungus in rows 1 and 2 are given in Table I. The types of lesions produced as a result of atrificial inoculation are shown in Plate 9, A. D.

TABLE I.—*Number of lesions on sugar beets inoculated with the sterile stage of Corticium vagum*

| Beet No. | Row 1, needle puncture. | Row 2, incision. |
|---|---|---|
| 1 | 23 | 8 |
| 2 | 7 | 0 |
| 3 | 11 | 3 |
| 4 | 13 | 13 |
| 5 | 8 | 8 |
| 6 | 8 | 2 |
| 7 | 17 | 6 |
| 8 | 14 | 2 |
| 9 | 11 | 6 |
| 10 | 17 | 4 |
| 11 | 6 | 4 |
| 12 | 17 | 6 |
| 13 | 13 | 7 |
| 14 | 17 | 8 |
| 15 | 20 | 3 |
| 16 | 19 | 2 |
| 17 | 10 | 6 |
| 18 | 15 | 4 |
| 19 | 11 | 4 |
| 20 | 12 | 0 |
| 21 | 16 | 0 |
| Total | 285 | 96 |
| Average | 13.5 | 4.5 |

Instructions for the inoculating of sugar beets with the beet fungus without puncture or incisions were not followed. As a result the question as to the ability of *Corticium vagum* to attack the sugar beet independently of other agents remains unsettled. It is quite conceivable that sugar-beet root aphis (*Pemphigus betae* Sloane) and other insects so prevalent in the soil may serve an important function in the initial entrance of the fungus. Having once gained access to the lower tissue, however, it appears evident from the results that this particular "strain" of *C. vagum* is capable of producing the type of canker and dryrot with which it is so constantly associated in the field.

The peculiar method of decay, together with the sharp line of demarcation between the diseased and the normal tissue (Pl. 8, A-C; 9, C-F), provide the most distinctive characteristics of the disease. A dark brown, watery layer invariably separates the dry, decayed mass occupying the cavity of the canker from the normal host tissue beneath. This layer

when examined under the microscope is found to be composed of masses of hyaline, vigorously growing young hyphae ramifying through and between the rapidly decaying host cells. It is in this advance layer that the major portion of the tissue destruction occurs. The brown layer advances uniformly inward by additions from the normal host tissue, while the outer surface of the layer rapidly dries out and constantly contributes its substance to the pithy mass occupying the resulting cavity of the canker. The thickness of the layer is dependent largely upon the rate at which the moisture is lost from it outer surface as the fungus eats its way radially into the normal tissue. No evidence of direct penetration of the normal cells by the fungus has been found. On the other hand, it appears that dissolving enzyms precede considerably the advancing mass of young hyphæ (Pl. 8, C).

This method of tissue destruction resembles in a very definite way that described by Ramsey (*5*), by which *Rhizoctonia solani* Kühn attacks and produces a definite pitting of the mature potato tuber. A similar process of decay is described by Atkinson (*1*) for the "sore shin" of cotton. He states that—

> the fungus (*Rhizoctonia solani*) never seems to penetrate far into the living tissues, but kills as it goes, and the tissues become brown, depressed, and present the appearance of a plant having a deep and ugly ulcer at the surface of the ground.

A type of decay most accurately resembling this particular beet rot is described by Richards (*7*) for the potato stem-canker caused by *Corticium vagum*.

The early production of definite cankers by a slow corroding of the normal tissue, finally resulting in a complete dryrot of the beet, suggests a possible name "dryrot canker" for the disease here described.

Various American workers. (*2*; *4, p. 243–54*; *3*) have reported rootrots of the sugar beet which they attribute to the work of *Rhizoctonia solani* Kühn. It appears difficult at this time, however, to determine the possible relation of these to the particular type of dryrot described in this article. The indefiniteness of the literature on the subject in fact does not justify any general statement as to the possible distribution of the disease.

During September and October of 1920 a preliminary survey [1] was made of the beet-growing districts in four counties of Utah—Cache, Davis, Utah, and Salt Lake. The disease was found in 18 fields of the 51 visited in Cache County and in 3 fields of the 20 surveyed in Davis County. Very serious damage occurred in a number of these fields. No indication of the trouble was found in either Utah or Salt Lake Counties.

The limited survey does not permit of an estimate of the loss to the total sugar-beet crop of the State; nevertheless, the general prevalence

[1] This survey was conducted in cooperation with the Office of Plant Disease Survey, United States Department of Agriculture. The author wishes to express his indebtedness to Dr. G. R. Lyman for this support.

of the trouble would indicate that under more favorable conditions the disease may become a serious factor in beet culture. It is not improbable that a thorough survey may discover the "dryrot canker" in every beet-growing district in this and surrounding States.

Since the appearance of the author's abstract (*6*), Dr. George L. Peltier reports in a letter to the author that he noted during 1920 in Nebraska what appears to be the same trouble. Preserved specimens in the plant-disease herbarium of the Utah Agricultural College show that the disease was collected in Utah as early as 1915.

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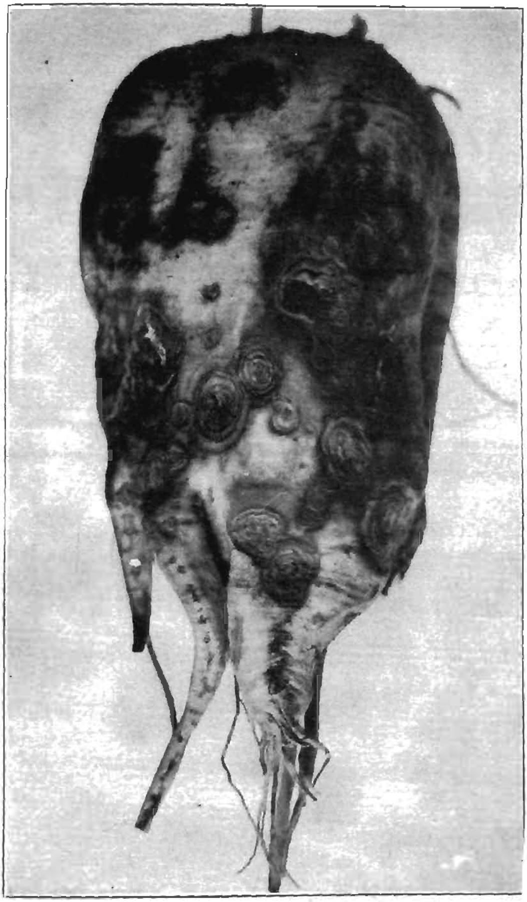
PLATE 4

Sugar beet showing typical lesions as a result of natural field infection. Lesions as shown may become confluent and develop common concentric rings. Initial stages in fissure formation are also evident. Photographed August 8, 1920.



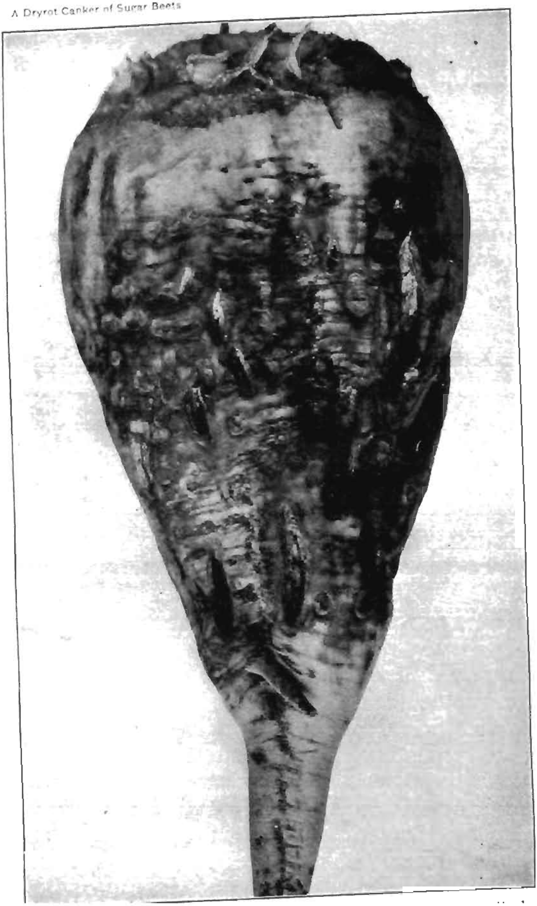
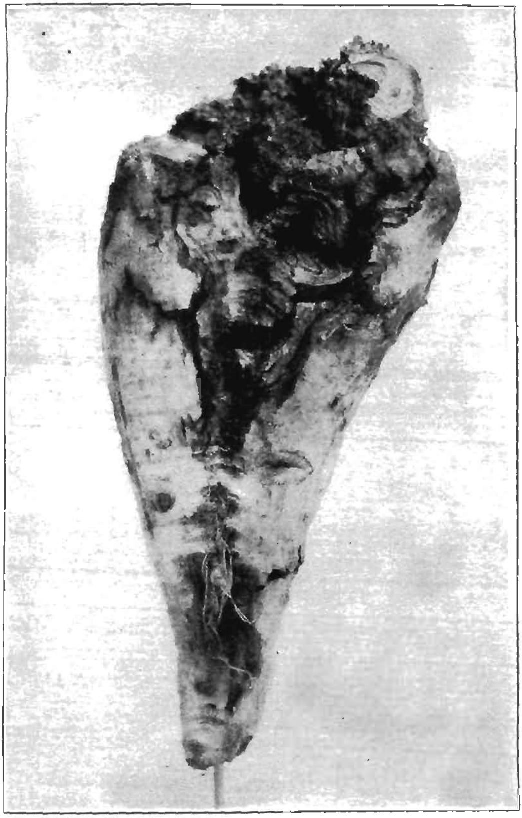


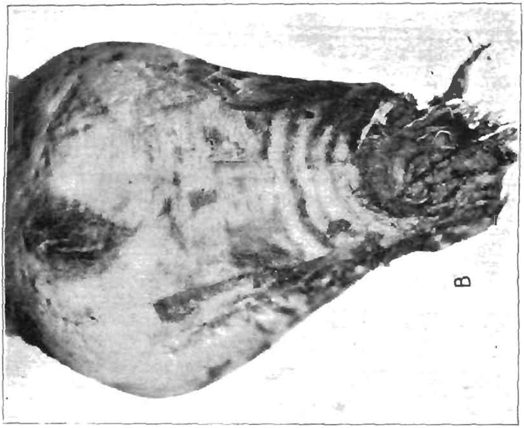
PLATE 5

Sugar beet showing various stages in the rupture of the outer covering of the lesion resulting in the formation of deep fissures. The lesions shown on this particular beet have not reached the size normally attained before rupture occurs.

## PLATE 6

*Late stage in the development of the disease, showing the beet as a dry shell partially filled with a pithy residue composed of mycelium and dead host tissue. The decay of the crown of this beet is a result of the fungus working upward from the point of infection below the surface of the soil. Remnants of the concentric rings of typical lesions are clearly visible. The cracking of the outer surface of the beet at this stage is shown to extend beyond the lesions.*

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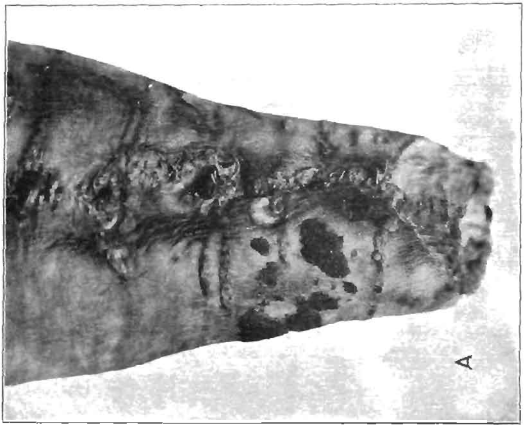
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PLATE 7

A.—Portion of a sugar beet showing the typical sclerotial masses commonly found adhering to the beets in the infested areas.

B.—Sugar beet showing the result of natural infection near the apex of the beet, at which point the root has been completely severed. The dryrot advancing upward from the initial point of attack has produced the typical undulating contour so characteristic of the small lateral lesions.

## PLATE 8

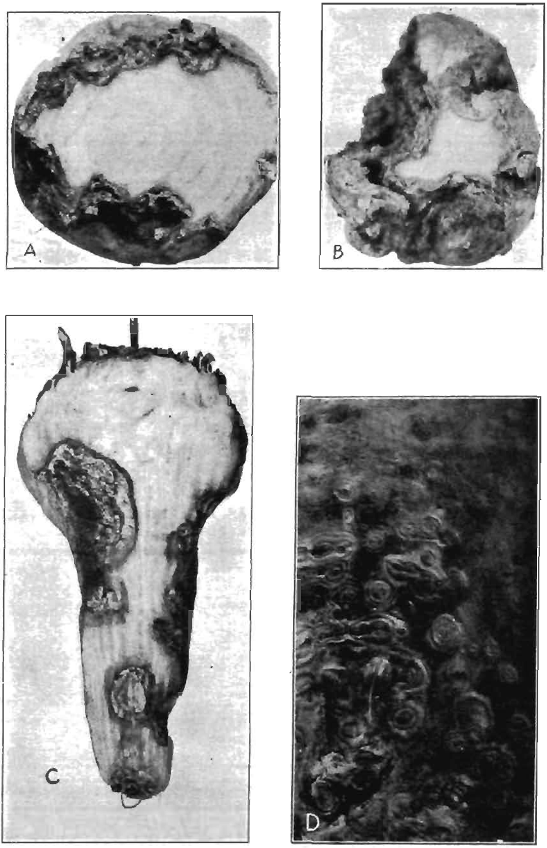
Sections of diseased sugar beets, showing the abrupt drying out between the diseased and healthy tissue. The prominent "feeding surfaces" composed of recently killed cells and the young hypha is clearly evident.

A.—Cross section, showing complete girdling of the beet by cankers resulting from separate points of infection. In such cases the continued penetration of the fungus may completely sever the root at the line of greatest infection.

B.—Longtitudinal section of diseased beet, showing various stages of decay and the pulpy material partially filling the cankers.

C.—Sugar-beet crown, showing the definite type of crownrot caused by the fungus worked upward from a point of infection below the soil surface (Pl. 6). A small region of healthy tissue is shown to which a few sickly leaves were attached.

D.—Section of beet surface, showing progressive stages in the development of the lesions resulting from natural infection. The earliest visible stage is shown to exhibit a slight perforation of the outer surface at the center of the lesion. This small opening, present in all lesions, gradually enlarges with age and finally results in the large fissures (Pl. 5). Various stages in the coalescence of lesions are especially evident.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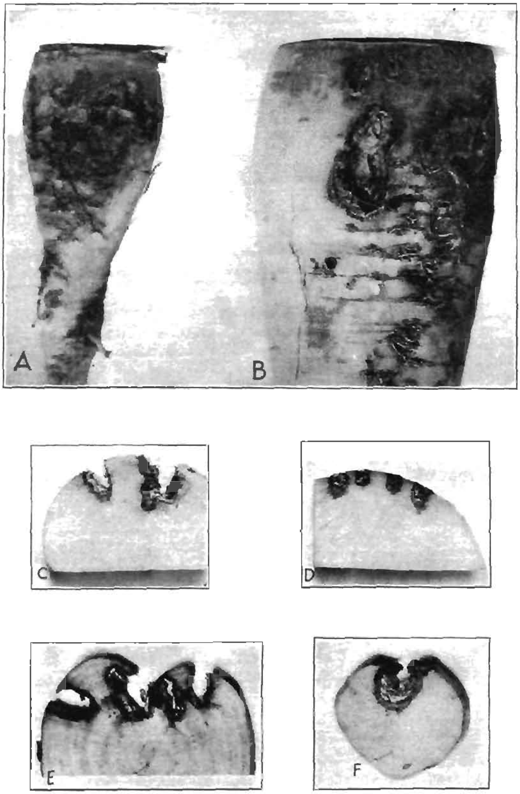
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PLATE 9

A, B.—Beets showing typical lesions produced by artificial inoculation. Needle punctures through which the fungus entered permitted of rapid drying out of the diseased tissue and of an early rupture of the outer layer of cells at the margin of the lesions. A number of the lesions, however, show the concentric rings so characteristic of the disease produced by natural infection. Cross sections of these lesions are shown in C and D.

C, D.—Cross sections of the lesions in A and B. The lesions in C disclose the more advanced stage of the disease wherein the outer layers of cells are broken down. In D the outer layers of the lesions are more or less entire.

E, F.—Cross sections of cankers resulting from natural infection in the field. A more advanced stage is shown than in C and D; otherwise the lesion produced by the natural and artificial method of inoculation appeared identical.

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## COMPARATIVE VIGOR OF $F_1$ WHEAT CROSSES AND THEIR PARENTS [1]

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The comparative vigor of $F_1$ crosses and their parents is a subject of much interest to the plant breeder. In crops where the technic of crossing is comparatively easy, the increase in vigor obtained in the $F_1$ cross often more than pays for the additional trouble of producing the hybrid seed. In self-fertilized crops like the small grains where considerable labor is involved in making artificial crosses, it is apparent that $F_1$ crosses can not be used commercially as a means of increasing crop yields. The suggestion, however, has been made by Anderson (*1*)[3] that the added vigor of the heterozygous condition might be utilized in small grains by making a large number of crosses between strains which, when crossed, show a considerable increase in yield. Produce from $F_2$ and $F_3$ progeny could be used for seeding the general field, and the crosses could be repeated each year in order to keep up the supply of seed.

Several theories have been advanced to explain the phenomenon of heterosis. The discovery of genetic linkage has led to the development of an adequate Mendelian explanation of the vigor so often obtained in $F_1$ crosses. An excellent review of the development of this theory is given by East and Jones (*8*). The theory explains the increase in vigor shown in the first hybrid generation as being due to the meeting in the zygote of dominant or partially dominant growth factors some of which are contributed by each parent. Linkage is given as the reason why all dominant factors can not be combined in a homozygous individual. According to this hypothesis the maximum number of favorable growth factors can be obtained only in the heterozygous condition.

In producing new varieties by crossing, forms may be obtained in the $F_3$ generation which appear homozygous for botanical and agronomic char-

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[3] Reference is made by number (italic) to "Literature cited," p. 61–63.

acters but which may be heterozygous for growth factors. There is the possibility that this heterozygous condition may cause the $F_2$ or F. hybrid to give a high yield. After several further generations this heterozygous condition may be lost, with a consequent loss in growth stimulus. A knowledge of the amount of added vigor in the $F_1$ generation is of value in determining whether heterozygosis in $F_3$ and $F_4$ lines would modify their yields sufficiently to interfere seriously with a determination of their value as improved varieties.

With these points in view a study has been made in wheat of the immediate effect of cross-pollination on seed weight and the increased vigor of $F_1$ crosses. Pure lines were used of seven varieties of *Triticum vulgare* Vill. and one variety of each *T. compactum* Host. (Little Club), *T. dicoccum* Schr. (Spring Emmer), and *T. durum* Desf. (Mindum). Varieties of *T. vulgare* were crossed with each other and with Little Club, Spring Emmer, and Mindum. Little Club was crossed also with Spring Emmer and Mindum.

## IMMEDIATE EFFECT OF CROSS-POLLINATION

Because of the phenomenon of double fertilization it is possible in some cases to obtain an increase in weight of seed as an immediate effect of cross-pollination. The increase is due principally to an increase in weight of endosperm in such crops as corn, where the proportion of endosperm to embryo is large.

Collins (5) observed open-pollinated ears of Chinese maize in which the size of seed was increased by cross-pollination. Seeds which showed by their color the effect of foreign pollen averaged 0.178 gm., while white seeds from the same portion of the ear averaged 0.153 gm. Roberts (*14*) mentions a similar instance with Chinese maize. Collins and Kempton (*6*) compared the average seed weight of corn from intravarietal and intervarietal pollinations. The intervarietal crosses exceeded the intravarietal in seed weight by 8.8 per cent. In a similar experiment, Wolfe (*10*) found that 23 of 31 corn varietal crosses yielded more grain than intravarietal pollinations. Carrier (*4*) obtained an increase in yield of grain in strains of corn when grown in a mixture as compared with any one of the strains grown alone.

That an increase is also obtained in the size of the embryo is clearly shown by Lewis and Vincent (*12*) in a comparison of seeds of Newtown apple from self- and cross-pollinations. The crossed seeds showed a striking increase in weight over that of the selfed seeds. As there is little or no endosperm in apple seeds, an increase in seed weight is due largely to an increased size of the cotyledons.

Since artificially pollinated seeds of wheat are usually smaller than normally pollinated seeds, spikes of each variety were emasculated in the same manner as for cross-pollination and then pollinated with pollen from plants of the same pure line. Seed from this intrapollination is

termed "incrossed seed" and is used as a basis of comparison in determining the immediate effect of cross-pollination. The average weight of a normally pollinated seed for all varieties used was 26.65 ± 0.22 mgm.,[1] and the average for an incrossed seeds was 18.13 ± 0.24 mgm.

A comparison is shown in Table I of the hybrid seed and the incrossed seed where the average dates of pollination are the same or approximately so.

TABLE I.—*Weight of seed of the immediate crosses compared with weight of seed of the incrossed parents*

| Name of cross. | Seed parent. | | Cross | | Difference between cross and female parent. |
|---|---|---|---|---|---|
| | Number of seeds. | Average weight of seeds. | Number of seeds. | Average weight of seeds. | |
| | | *Mgm.* | | *Mgm.* | *Mgm.* |
| Marquis × Velvet Chaff[a] .... | 38 | 12.6±0.5 | 48 | 15.6±0.5 | +3.0±0.7 |
| Marquis × Penny | 38 | 12.6± .5 | 24 | 20.2±1.0 | +7.6±1.2 |
| Haynes Bluestem × Marquis | 49 | 17.2± .8 | 26 | 23.5± .7 | +6.3±1.0 |
| Little Club × Marquis ....... | 39 | 10.1± .5 | 50 | 9.4± .3 | −0.7± .6 |
| Emmer × Velvet Chaff.... | 44 | 26.4± .8 | 24 | 27.1±1.3 | +0.7±1.5 |
| Velvet Chaff × Mindum .. | 104 | 19.9± .6 | 23 | 15.9± .6 | −4.0± .8 |
| Emmer × Little Club .. | 44 | 26.4± .8 | 15 | 25.0±1.2 | −1.4±1.4 |

[a] In the discussion of crosses the seed parent is given first.

The varietal crosses in every case showed an increased seed weight as compared with the female parent. The largest increase in seed weight was 7.6 ± 1.2 mgm., which was obtained from the cross Marquis × Penny. This hybrid gave on the average over 50 per cent heavier seeds than incrossed Marquis. Of the species crosses none gave a significant increase in seed weight. Velvet Chaff crossed with Mindum produced seeds which on the average were 4.0 ± 0.8 mgm. lighter than the seeds of incrossed Velvet Chaff.

## $F_1$ GENERATION CROSSES COMPARED WITH THEIR PARENTS

Some of the earliest hybridization work affords good examples of the vigor of $F_1$ crosses. For an excellent review of this subject the reader is referred to the publication of East and Jones (*8*).

In the present experiment the $F_1$ generations and their parents were grown in the greenhouse under controlled conditions. Care was taken to plant seeds at a uniform depth, and when the seedlings were about 4 inches tall they were transplanted to 7-inch pots, two seedlings to a pot and only like seedlings together. Unfortunately an epidemic of stem-rust

[1] The probable error of an average of averages was calculated according to the formula:

$$E=\frac{1}{N}\sqrt{n_1^2e_1^2+n_2^2e_2^2 - - - - n_n^2e_n^2},$$

in which $n$ is the number of individuals in a generation, $e$ the probable error, and $N$ the total number of individuals (*12*).

started about heading time, and some plants were rusted badly Measurements of height were taken on those plants which were not attacked previous to heading, and yield data were taken only on plants uninjured by rust.

Both incrossed and normally pollinated seeds of the parental varieties were planted. Arny and Garber (2) have shown that in some cases there is a positive correlation between weight of seed planted and the vigor of resultant plants. In order to determine whether the size of seed planted was of importance in an analysis of individual plant yields in the present experiment, correlation coefficients were calculated for the weight of seed planted as subject and length of culm and yield of grain per plant as relative. (Table II.)

TABLE II.—*Correlation coefficients for weight of seed planted and the vigor of resultant plants*

| Variety. | Coefficient of correlation with weight of seed planted as subject. | | |
|---|---|---|---|
| | Length of tallest culm (relative). | Total culm length (relative) | Yield of grain in grams per plant (relative). |
| Marquis | −0.084±0.100 | +0.136±0.098 | +0.047±0.173 |
| Velvet Chaff | + .069± .065 | + .078± .065 | + .087± .108 |
| Barletta | − .144± .085 | .205± .083 | .............. |
| Penny | − .197± .075 | − .129± .077 | + .010± .112 |
| H. B. S. 1-16-12 | − .242± .071 | + .121± .071 | − .194± .095 |
| Bobs | − .116± .079 | + .169± .078 | + .205± .084 |
| Little Club | − .118± .082 | + .185± .080 | + .032± .099 |
| Emmer | + .024± .086 | − .007± .085 | − .425± .080 |
| Mindum | − .121± .076 | + .381± .066 | + .136± .095 |

The only significant correlation was obtained with the Mindum variety. A correlation coefficient of $+0.381 \pm 0.066$ was obtained for weight of seed planted and total culm length. In the light of these facts it was considered legitimate to use the plants from normal and incrossed seed as a single parent population.

The $F_1$ crosses and their parents were compared for height of tallest culm and for total culm length. (Table III.)

Six of the 11 $F_1$ varietal crosses showed an increase in length of tallest culm as compared with the parental average, and 5 showed a decrease. The two $F_1$ crosses between Mindum and vulgare varieties were considerably taller than either parent. Similar results were obtained from crosses between Emmer and the same vulgare varieties. On the other hand, the $F_1$ crosses of Little Club with either Emmer or Mindum did not show a significant difference in average height of tallest culm when compared with the average of the parents.

In Table IV the crosses and their parents are compared for total culm length.

TABLE III.—*Height of tallest culm of $F_1$ wheat crosses compared with parental average*

| Name of one parent. | Number of individuals. | Height. | Name of other parent | Number of individuals. | Height. | Average height of parents. | $F_1$ cross. Number of individuals. | $F_1$ cross. Height. |
|---|---|---|---|---|---|---|---|---|
| | | *Inches.* | | | *Inches.* | *Inches.* | | *Inches.* |
| Marquis | 46 | 46.5 | Velvet chaff | 105 | 44.7 | 45.6 | 64 | 42.3 |
| | 46 | 46.5 | Barletta | 60 | 48.7 | 47.6 | 38 | 50.8 |
| | 46 | 46.5 | Penny | 74 | 37.4 | 41.9 | 45 | 41.1 |
| | 46 | 46.5 | Bobs | 70 | 40.1 | 43.3 | 65 | 47.9 |
| Velvet Chaff | 105 | 44.7 | Barletta | 60 | 48.7 | 46.7 | 38 | 50.9 |
| | 105 | 44.7 | Penny | 74 | 37.4 | 41.0 | 49 | 42.6 |
| | 105 | 44.7 | Bobs | 70 | 40.1 | 42.4 | 108 | 40.7 |
| Penny | 74 | 37.4 | Bobs | 70 | 40.1 | 38.7 | 60 | 33.6 |
| Haynes Bluestem | 79 | 50.8 | Marquis | 46 | 46.5 | 48.6 | 24 | 50.8 |
| Marquis | 46 | 46.5 | Little Club | 66 | 49.2 | 47.8 | 62 | 50.5 |
| Velvet Chaff | 105 | 44.7 | Little Club | 66 | 49.2 | 46.9 | 52 | 43.3 |
| Average | 73 | 45.4 | | 69 | 43.8 | 44.6 | 55 | 45.0 |
| Emmer | 62 | 51.9 | Little Club | 66 | 49.2 | 50.5 | 12 | 51.0 |
| Mindum | 77 | 49.7 | Little Club | 66 | 49.2 | 49.4 | 2 | 48.5 |
| | 77 | 49.7 | Marquis | 46 | 46.5 | 48.1 | 14 | 53.2 |
| | 77 | 49.7 | Velvet Chaff | 105 | 44.7 | 47.2 | 17 | 54.5 |
| Emmer | 62 | 51.9 | Velvet Chaff | 105 | 44.7 | 48.3 | 28 | 55.5 |
| | 62 | 51.9 | Marquis | 46 | 46.5 | 49.2 | 18 | 55.6 |
| Average | 70 | 50.8 | | 72 | 46.8 | 48.8 | 15 | 53.1 |

TABLE IV.—*Total culm length of $F_1$ wheat crosses compared with parental average*

| Name of one parent | Number of individuals. | Height. | Name of other parent. | Number of individuals. | Height. | Average height of parents. | $F_1$ cross. Number of individuals. | $F_1$ cross. Height. |
|---|---|---|---|---|---|---|---|---|
| | | *Inches.* | | | *Inches.* | *Inches.* | | *Inches.* |
| Marquis | 46 | 195 | Velvet Chaff | 105 | 149 | 172 | 64 | 146 |
| | 46 | 195 | Barletta | 60 | 157 | 176 | 38 | 168 |
| | 46 | 195 | Penny | 74 | 90 | 143 | 45 | 132 |
| | 46 | 195 | Bobs | 70 | 136 | 166 | 65 | 211 |
| | 46 | 195 | Little Club | 66 | 149 | 172 | 62 | 167 |
| Velvet Chaff | 105 | 149 | Barletta | 60 | 157 | 153 | 38 | 174 |
| | 105 | 149 | Penny | 74 | 90 | 120 | 49 | 133 |
| | 105 | 149 | Bobs | 70 | 136 | 143 | 108 | 153 |
| | 105 | 149 | Little Club | 66 | 149 | 149 | 52 | 153 |
| Penny | 74 | 90 | Bobs | 70 | 136 | 113 | 60 | 111 |
| Haynes Bluestem | 79 | 215 | Marquis | 46 | 195 | 205 | 24 | 214 |
| Average | 73 | 171 | | 69 | 140 | 156 | 55 | 160 |
| Emmer | 62 | 204 | Little Club | 66 | 149 | 177 | 12 | 169 |
| Mindum | 77 | 131 | Little Club | 66 | 149 | 140 | 2 | 189 |
| | 77 | 131 | Marquis | 46 | 195 | 163 | 14 | 151 |
| | 77 | 131 | Velvet Chaff | 105 | 149 | 140 | 17 | 123 |
| Marquis | 46 | 195 | Emmer | 62 | 204 | 200 | 18 | 210 |
| Velvet Chaff | 105 | 149 | Emmer | 62 | 204 | 177 | 28 | 149 |
| Average | 74 | 157 | | 68 | 175 | 166 | 15 | 165 |

For total culm length, 6 of the 11 varietal crosses showed an increase over the parental average and 5 showed a decrease. The averages for culm length of the $F_1$ crosses and of their parents are practically identical when the results of all crosses are considered together. This makes it doubtful whether the increases of the $F_1$ crosses over the parental averages are the results of the vigor due to crossing or are due to some other experimental factor.

TABLE V.—*Average yield of grain per plant of $F_1$ wheat crosses and their parents*

| Name of one parent. | Number of individuals. | Yield. | Name of other parent | Number of individuals. | Yield. | Average yield of parents. | $F_1$ cross: Number of individuals. | $F_1$ cross: Yield. | Percentage of increase with parental average as basis. |
|---|---|---|---|---|---|---|---|---|---|
| | | *Gm.* | | | *Gm.* | *Gm.* | | *Gm.* | |
| Marquis | 15 | 1.9 | Penny | 36 | 2.4 | 2.2 | 18 | 2.7 | 33 |
| | 15 | 1.9 | Bobs | 59 | 3.0 | 2.5 | 65 | 3.3 | 32 |
| Velvet Chaff | 38 | 1.5 | Penny | 36 | 2.4 | 2.0 | 28 | 2.5 | 25 |
| | 38 | 1.5 | Bobs | 59 | 3.0 | 2.3 | 02 | 2.9 | 26 |
| Penny | 36 | 2.4 | | 59 | 3.0 | 2.7 | 23 | 2.8 | 4 |
| Haynes Bluestem | 47 | 2.4 | Marquis | 15 | 1.9 | 2.2 | 18 | 2.5 | 14 |
| Marquis | 15 | 1.9 | Little Club | 46 | 2.2 | 2.1 | 45 | 2.3 | 10 |
| Velvet Chaff | 38 | 1.5 | | 46 | 2.2 | 1.9 | 37 | 2.5 | 32 |
| Average | 30 | 1.9 | | 45 | 2.5 | 2.2 | 41 | 2.7 | 23 |
| Little Club | 46 | 2.2 | Emmer | 48 | 1.1 | 1.7 | 9 | .3 | ..... |
| | 46 | 2.2 | Mindum | 49 | 2.1 | 2.2 | 1 | 1.0 | ..... |
| Marquis | 15 | 1.9 | | 49 | 2.1 | 2.0 | 13 | .3 | ..... |
| Velvet Chaff | 38 | 1.5 | | 49 | 2.1 | 1.8 | 8 | 1.1 | ..... |
| | 38 | 1.5 | Emmer | 48 | 1.1 | 1.3 | 23 | .5 | ..... |
| Marquis | 15 | 1.9 | | 48 | 1.1 | 1.5 | 18 | .6 | ..... |
| Average | 33 | 1.9 | | 49 | 1.6 | 1.8 | 12 | .6 | ..... |

For average yield of grain per plant, six of the eight variety crosses yielded more than either parent, and all variety crosses yielded more than the parental average. Marquis × Bobs and Velvet Chaff × Little Club exceeded the parental average 32 per cent in yield of grain per plant.

With the exception of crosses between common wheat and Little Club the average yield of grain per plant of the species crosses was less than that of the lower-yielding parent. This is due to the fact that the $F_1$ plants had a high percentage of barren florets.

## STERILITY IN SPECIFIC CROSSES

The occurrence of sterility in wheat specific crosses has been reported by several workers. Tschermak (*15*), after several years of hybridization work, found that hybrids of ***Triticum dicoccum*** and ***T. compactum*** or vulgare varieties were only partially fertile. Hybrids of ***T. durum*** with ***T. compactum*** or ***T. vulgare*** varieties were classed as fully fertile. Ster-

ility is mentioned by Kezer and Boyack (*11*) as occurring in the $F_1$ generation of the cross Fultz Mediterranean by Black Winter Emmer. In crosses between Algerian Macaroni and Algerian bread wheats, Freeman (*9*) reports that the $F_1$ generation developed normally but in the $F_2$ generation all degrees of sterility appeared from complete sterility to complete fertility.

Hayes, Parker, and Kurtzweil (*10*) crossed varieties of *Triticum vulgare* with varieties of *T. durum* and *T. dicoccum*. The parental varieties showed an average of 4 per cent of barren florets. The $F_1$ crosses of varieties of durum with varieties of vulgare and the reciprocals showed a barrenness of 47 per cent. The $F_1$ crosses of *T. dicoccum* crossed with varieties of vulgare showed 26 per cent barrenness and the reciprocal 29 per cent. The results are not in agreement with the conclusions of Tschermak (*15*).

In the present experiment a count was made of the total number of outer florets per plant and the number of these which were barren. From these data the percentage of barren florets was computed. (Table VI.)

TABLE VI.—*Barrenness of outer florets in wheat varieties and $F_1$ crosses*

| Variety or cross. | Number plants considered. | Percentage of barren outer florets. |
|---|---|---|
| Marquis | 15 | 18 |
| Velvet Chaff | 38 | 21 |
| Penny | 34 | 15 |
| Haynes Bluestem | 47 | 18 |
| Bobs | 59 | 17 |
| Little Club | 47 | 25 |
| Emmer | 49 | 18 |
| Mindum | 44 | 19 |
| Average | 42 | 19 |
| Marquis × Penny | 18 | 17 |
| Marquis × Bobs | 65 | 14 |
| Velvet Chaff × Penny | 27 | 15 |
| Velvet Chaff × Bobs | 93 | 13 |
| Haynes Bluestem × Marquis | 18 | 17 |
| Penny × Bobs | 22 | 15 |
| Marquis × Little Club | 57 | 17 |
| Velvet Chaff × Little Club | 37 | 14 |
| Average | 42 | 15 |
| Marquis × Emmer | 18 | 73 |
| Velvet Chaff × Emmer | 23 | 67 |
| Little Club × Emmer | 10 | 86 |
| Average | 17 | 75 |
| Marquis × Mindum | 13 | 88 |
| Velvet Chaff × Mindum | 8 | 67 |
| Little Club × Mindum | 2 | 54 |
| Average | 8 | 70 |

The parental varieties showed an average of 19 per cent barren florets. Intercrosses of vulgare varieties and crosses between Little Club and vulgare varieties showed an average of 15 per cent barren florets. The $F_1$ crosses of Marquis, Velvet Chaff, and Little Club with Emmer gave an average of 75 per cent barrenness. These same common varieties and Little Club crossed with Mindum showed a barrenness of 70 per cent. These data confirm the results of Hayes, Parker, and Kurtzweil (*10*) and show conclusively that in some cases $F_1$ crosses between varieties of *T. vulgare* and *T. durum* or *T. dicoccum* are highly self-sterile.

## DISCUSSION OF RESULTS

It has been pointed out by East and Jones (7) that the increase in productivity of a cross is due to an increase in the number of growth factors of which the maximum number can be obtained only in a heterozygous condition. In a crop such as corn, this heterozygous condition is kept up by cross-fertilization. Selfing corn varieties reduces the heterozygosity and consequently the vigor. In wheat the continued selfing natural to the crop has brought about a condition of homozygosity.

In the present experiment all varietal crosses gave an increase in seed weight as an immediate effect of cross-pollination. An increase is also shown in the $F_1$ crosses for average yield of grain per plant as compared with the parental average. The increase ranged from 4 per cent in Penny × Bobs to 32 per cent in Marquis × Bobs. Before attempting to utilize the vigor of the heterozygous condition by growing $F_3$ and $F_4$ generation crosses as the commercial crop, it seems logical to combine in one variety the maximum number of growth factors possible. When the possibilities of combination have been exhausted and a variety, or a series of varieties, has been secured which contains this maximum number of growth factors, it may be desirable to follow out the suggestion of Anderson (*1*). This method probably could not be used to advantage except under intensive farming conditions. In case one desired to use such a method it is logical to assume that the more desirable crosses to make are those which show the greatest increase in yield of grain in the $F_1$ generation.

There is an indication that the increased productivity of the heterozygous condition is a factor which must be considered in comparing $F_3$ and $F_4$ lines for yielding ability. In the cross Marquis × Bobs the $F_1$ generation showed on an average a 32 per cent increased yield of grain per plant as compared with the average of the parents and a 10 per cent increase as compared with the higher-yielding parent. In the $F_2$ generation of such a cross it is highly probable that some of the most vigorous plants will be those with the greatest degree of heterozygosity. These heterozygous individuals will produce $F_3$ progeny the vigor of which likewise will be partially due to the heterozygous condition. If the $F_3$ lines are classified on a basis of their yielding ability, some of these heterozygous lines will

be included as the best yielders. In subsequent generations as the lines become homozygous their productivity may decrease. In generations beyond the $F_6$ the heterozygous condition of the population rapidly disappears.

A method of breeding which, according to Babcock and Clausen (*3*), has been used by the Svalöf Station, seems worthy of wider application. As self-fertilized crops approach homozygosis rapidly in generations following a cross, it is suggested that a cross be made between varieties selected because of the desirable characters which they possess. After 6 to 10 years have elapsed, during which time progeny of the cross has been grown in bulk plots, selection of individual plants may be made with the assurance that a high percentage of these plants will give homozygous progeny. While this system requires some length of time before results are obtained, it requires a minimum of labor.

When making crosses with the hope of increasing yield through a recombination of the desirable factors of both parents, the parents will naturally be selected on the basis of their yielding ability. The chances of favorable recombinations of yield factors in generations following a cross will presumably be greater when dealing with a cross which shows maximum increased yield over the parents in the $F_1$ generation. Genetic linkage, however, may make certain combinations difficult or impossible.

The sterility of the specific crosses, with the exception of crosses of varieties of *Triticum vulgare* with Little Club, is partially or wholly responsible for the low grain yield of the crosses as compared with the parental averages. The fact that Little Club behaves in every way as a variety of *T. vulgare* agrees with the view of Tschermak (*15*), who believes that *T. compactum* and *T. vulgare* are closely related. Little Club crosses readily with varieties of vulgare. When Little Club or varieties of vulgare are crossed with Emmer or Mindum, the same high degree of sterility is shown.

## SUMMARY OF RESULTS

(1) An increase in seed weight was obtained in all varietal crosses as an immediate effect of cross-pollination. The only significant difference shown by the immediate hybrids of specific crosses was a decrease in seed weight obtained in Velvet Chaff × Mindum.

(2) In the $F_1$ generation some of the hybrids exceeded the parental average in height of tallest culm, and in total culm length others showed a decrease. In all varietal crosses the $F_1$ hybrid exceeded the parental average in yield of grain per plant, and six out of eight crosses exceeded the yield of the better parent.

(3) Crosses between Little Club and varieties of *Triticum vulgare* gave results similar to those of crosses between vulgare varieties.

(4) The $F_1$ generation of Emmer or Mindum crossed with varieties of *Triticum vulgare* or with Little Club showed a high degree of sterility.

The average percentage of barren florets of the parental varieties was 19. The average percentage of barren florets of the $F_1$ varietal crosses, including crosses of Little Club with vulgare varieties, was 15. The vulgare-Emmer and Little Club-Emmer crosses produced 75 per cent barren florets, while an average of 70 per cent of barren florets was obtained from the durum-vulgare and durum-Little Club crosses.

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# TEMPERATURE AND HUMIDITY STUDIES OF SOME FUSARIA ROTS OF THE IRISH POTATO [1]

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## INTRODUCTION

The ability of *Fusarium oxysporum* Schlect. to cause a rot of the potato tuber has been clearly demonstrated by a number of workers. The influence of temperature on this disease has been reported in a number of papers, but the experimental evidence as a whole is rather meager, usually only extreme temperatures being used. The effect of moisture on the progress of the disease, except under conditions of extreme dryness or saturation, has received practically no attention. It was with the purpose of determining the relation of temperature and humidity to the progress of potato tuber-rots caused by Fusaria that the following work was undertaken.

## HISTORICAL

The association of Fusaria with storage-rots of the Irish potato (*Solanum tuberosum* L.) has been a matter of common observation by most workers in plant pathology from 1842 to date. Several species of the form genus Fusarium Link have been described as causes of potato tuber-rots, by Von Martius (*12*), Reinke and Berthold (*18*), Schacht (*19*), Pethybridge and Bowers (*14*),[2] Longman (*10*), and Sherbakoff (*20*). The fact that Fusarium species could produce a rot of the tuber was demonstrated by Pizzigoni (*15*) and Wehmer (*24*, *25*), who described the species they worked with as *Fusarium solani* (Mart). Frank (*6*), De Bary (*2*), and others considered that the Fusaria were unable to produce a rot of the tuber. In most of the earlier papers, *F. solani*, or some species thought to be a synonym of it, was given as the causal organism.

Owing to the absence of clearly defined species in all the literature previous to Appel and Wollenweber's (*1*) monograph on the form genus Fusarium in 1912, no attempt will be made to review in detail the earlier reports of potato tuber-rots caused by Fusaria.

*Fusarium oxysporum* was considered by Wollenweber (*27*) to be a strictly vascular parasite producing a wilt of the potato vine but not

[1] Published with the approval of the Director of the Nebraska Agricultural Experiment Station. The paper is based upon experimental work undertaken at the Michigan Agricultural College in 1914–15, and at the University of Wisconsin in 1916–17.

[2] Reference is made by number (italic) to "Literature cited," p. 77-79.

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causing a rot of the tuber. Carpenter (*4*) in 1915 was the first to report successful infections by inoculations with pure cultures of *F. oxysporum*. He made these by dipping wounded tubers in a water suspension of spores, wrapping in oiled paper and keeping them at controlled temperatures ranging between 17° and 30° C. No detailed experiments were reported except in this saturated atmosphere. He noted, however, that either a dryrot or a wetrot was produced, according to the temperature and humidity used. He concluded that a constant storage temperature below 50° F. (10° C.) would prevent the action of *F. radicicola* Wollenw., *F. eumartii* Carp., and *F. oxysporum*. Previous to this work of Carpenter's, Smith and Swingle (*22*), in 1910, described a bundle blackening and a dry endrot of the tuber as two stages of the same disease. They attributed this to a Fusarium for which they accepted the name *F. oxysporum* as first applied to it by Schlechtendahl (*21, p. 139*). They noted that the disease continued in stored potatoes and that when potatoes were stored in warm rooms, either moist or dry, they became badly diseased, whereas those stored in cool places kept much better. They did not differentiate this species of Fusarium from others occurring on the potato, and no inoculation experiments were recorded. Manns in 1911 (*11*), working with the same disease, stated that the "dormant internal infection" under improper storage conditions becomes so active as to cause a high percentage of dryrot. He noted that the disease was favored by high temperature and considerable moisture. At 36° to 40° F. (2° to 3° C.) the disease made no progress, at 45° to 55° F. (7° to 12° C.) it developed gradually and caused considerable rot, especially when accompanied by high humidity. He made no mention of pure culture inoculations on tubers or morphological studies.

Jamieson and Wollenweber in 1912 (*8*) described a dryrot of the potato tuber caused by a species of Fusarium which they named *Fusarium trichothecioides* Wollenw. They made inoculation experiments and found the most rapid penetration of the tuber to take place at 10° to 12° C. in an atmosphere of low humidity. Rotting took place at the high humidities but not as rapidly. Wilcox, Link, and Poole (*26*) published on a dryrot of the potato tuber caused by a Fusarium which they called *F. tuberivorum* W. and L. but which was undoubtedly the *F. trichothecioides* previously described by Jamieson and Wollenweber (*8*). They found that a temperature of 8° to 10° C. was only slightly inhibitive to the growth of the fungus and that when potatoes infected with the organism were stored at this temperature, the most rapid decay took place when the humidity was high. Pratt (*17*), working with the same disease, found that temperatures ranging from 12° to 25° C. were favorable for the progress of the disease and that dryrot did not develop at temperatures below 2° C. He concluded from storage experiments that in a dry, well-ventilated storage house losses would be very slight at temperatures from 2° to 4° C.

Link (*9*), making comparative studies of *Fusarium oxysporum* and *F. trichothecioides* found that both were capable of producing a rot of the potato tuber and that *F. trichothecioides* produced a typical dry-rot. *F. oxysporum* produced a softrot of the whole tuber except under cold, dry conditions, when a dryrot was produced. He ran his experiments at controlled temperatures ranging from 1° to 30° C. in an almost saturated atmosphere.

Pratt (*16*) found that *Fusarium radicicola* behaved much the same as *F. oxysporum*, and he concluded from storage experiments that the tuber-rot caused by this organism does not make any progress in storage at a temperature of 48° F. (8.8° C.) or below.

In general, then, it can be said that a high temperature favors the production of tuber-rots by all three of these Fusaria, although *Fusarium trichothecioides* appears to be able to produce a rot at lower temperatures than the other two. High humidities also appear to favor the production of tuber-rot. With the exception of the paper of Jamieson and Wollenweber (*8*) all the evidence points toward an increase in rotting with an increase in humidity.

## TEMPERATURE RELATIONS IN PURE CULTURES

A review of the literature shows a general conformity of results regarding the relation of temperature to the growth of *Fusarium oxysporum*. Link (*9*) by making dry-weight determinations of growth in liquid media found 30° C. to be the optimum for growth. Edson and Shapovalov (*5*), working with Petri-dish cultures, obtained the same optimum. They reported a maximum temperature of 37 C., where the spores changed to chlamydospores; they did not observe growth at 5° C. Humphrey (*7*) gives 4° C. as the minimum temperature for certain strains of *F. oxysporum*.

The writer, working with three strains of *Fusarium oxysporum* and using the same methods for measuring growth, obtained somewhat similar results to those reported by Edson and Shapovalov (*5*). The minimum temperature for growth was 9.5° C., no growth taking place at the next lower temperature of 7° C. The maximum temperature was 37.5° C., where there was a very slight growth.

*Fusarium trichothecioides* is apparently unable to grow at 30° C., which is the optimum temperature for *F. oxysporum*. Link (*9*) found the greatest growth of *F. trichothecioides* in liquid potato extract media at the end of 20 days to take place at 12° C., with no growth present at 30° C., although the organism was capable of living in the potato tuber at that temperature. Edson and Shapovalov (*5*) obtained a much higher optimum for *F. trichothecioides;* they found the greatest growth took place at 25° C., with a sharp drop to the maximum temperature at 30° C., where germination of spores took place but no growth of mycelium.

The writer, working with two strains of *Fusarium trichothecioides* in Petri dishes, found 25° C. to be the optimum temperature, and with one strain he was able to obtain slight growth, 7 mm. in diameter, at the end of one week at 30° C. At 5° C. germination took place and there was slight growth.

The optimum temperature for *Fusarium radicicola* was 30° C., the same as for *F. oxysporum*. The minimum was at 5° C., where a very slight growth was produced in 10 days. At 35° C. the growth was greater than with *F. oxysporum*, although the rate of growth was slower. Edson and Shapovalov (5) report a similar optimum temperature, with germination but no growth at 5° C. They found that at 39° C. a transformation from normal spores to chlamydospores took place.

In general it can be said that at 25° C. the growth for all three species is nearly equal, *Fusarium oxysporum* and *F. radicicola* increasing in growth up to 30° C. and *F. trichothecioides* decreasing. The minimum temperature for *F. oxysporum* is higher than for the other two, and in general *F. trichothecioides* appears to be more tolerant of the lower temperatures than the others.

Preliminary experiments, using liquid media and determining the growth by dry weights, have been conducted with a number of strains of these three species. While on certain media the results have in general corroborated the foregoing cardinal points for growth, they indicated that these cardinal points may vary with the medium used. For instance, with an nutrient solution made up of ammonium nitrate ($NH_4NO_3$), potassium phosphate ($KH_2PO_4$), magnesium sulphate ($MgSO_4$), ferric chlorid ($FeCl_3$), and sucrose, the results compared well with those obtained on agar in Petri dishes. With a nutrient solution made up similarly to the potato extract medium used by Link (9), the total growth at the higher temperatures was considerably less than the growth obtained in the first nutrient solution, while at the lower temperatures the growth was much greater. The optimum temperature for growth of *Fusarium trichothecioides* in the first nutrient solution was 25° C., with no growth taking place at 5° C. With Link's potato-extract medium the optimum lay between 15° and 20° C., and there was weighable growth at 5° C. These results would possibly account for the considerable discrepancy between the results obtained by Link (9) with liquid media and those obtained by the writer and by Edson and Shapovalov (5) with agar cultures.

## EXPERIMENTAL INFECTION OF TUBERS

The cultures used in the following experiments, with their origin, are listed below. In practically all cases the various strains of the same species behaved alike. Several other strains of *Fusarium oxysporum*, isolated by the writer, were also used in the experiments in addition to the ones listed below.

No. 1.—*Fusarium oxysporum*, isolated by the author from browned vascular bundles of potatoes and identified by H. W. Wollenweber and numbered at Washington as 3377.

No. 8. *Fusarium oxysporum*, obtained from C. W. Carpenter of the United States Department of Agriculture, No. 3395.

No. 32.—*Fusarium oxysporum*, obtained from G. K. K. Link, of the University of Nebraska, as No. 3345a.

No. 28.—*Fusarium trichothecioides* obtained from G. K. K. Link.

No. 31.—*Fusarium trichothecioides*, obtained from A. C. Pratt of the United States Department of Agriculture.

No. 29.—*Fusarium radicicola*, obtained from A. C. Pratt and numbered 716.

## METHODS

In all inoculation experiments with tubers, potatoes which were of one variety, of the same age, and had been kept under the same storage conditions were carefully selected for uniformity of size, type, and freedom from wounds. The stem ends always were cut and examined for natural infection, and all tubers showing vascular discoloration were discarded. The tubers were always treated with formaldehyde or mercuric chlorid and washed in sterile distilled water.

The inoculations were made by wounding the epidermis, usually by stabbing to a depth of 3 mm. with a sterile scalpel. The inoculum was introduced in various ways as outlined in the experiments.

Experiment 1, December, 1915.—Potato tubers of the Up-to-Date variety were inoculated by wounding the tubers and then dipping them in a water suspension of spores, wrapping in sterile waxed paper, and placing in moist chambers at 25° C. Controls were treated in the same way, being dipped in sterile water. Results were taken 18 days later.

Set No. 1. Four tubers inoculated with *Fusarium oxysporum* No. 1. All tubers completely rotted. The two control tubers remained sound.

Set No. 2. Four tubers inoculated with *Fusarium oxysporum* isolated from infected tubers in storage. All tubers showed a complete wetrot; the tissue was soft and of a light brown color; a large cavity was present in each tuber containing masses of white mycelium. At the point of inoculation there was a granular mass of hyphae and starch grains separated from the rest of the tissue. Control tubers remained healthy.

Set No. 3. Four tubers inoculated with *Fusarium oxysporum*, isolated from wilted potato vines. All tubers showed a dark brown dryrot progressing only a short distance from the point of inoculation. Controls remained healthy.

Reisolations were made from all the rotted tubers, and *Fusarium oxysporum* was recovered in every case. No bacteria or secondary invaders were found in any of the tubers. These results show that *F. oxysporum* is capable of producing a rot of the tuber in a saturated atmosphere at

25° C. The characteristic rot under these conditions is a soft wetrot with no sharp line of demarkation between the healthy and diseased tissue. The organism appears to be unable to attack whole starch grains, which accumulate in a granular mass with the mycelium, as in set 2. The tuber-rot under these abnormal conditions is not typical of the rots usually found in storage.

EXPERIMENT 2, FEBRUARY 23, 1916.—Further tests were conducted at the same temperature but with a lower relative humidity to test the ability of the organism to cause a rot under conditions not so adverse for the host as in the previous experiment. Tubers of the Up-to-Date variety were inoculated by wounding and then placing a little of the fungus mycelium and spores in the wound. The tubers were then placed in a sterile moist chamber but were not wrapped in paper. Controls were treated and wounded in the same way. The experiment was run at 25° C. Twelve different strains of *Fusarium oxysporum* were used for the inoculations, two tubers being used for each strain. Results were taken after five weeks.

In only one case had the rot extended three-fourths of the length of the tuber. In all the other tubers there was only a slight rotting extending for a short distance from the point of inoculation. The controls remained sound in every case. The tubers were in a saturated atmosphere at the beginning of the experiment, gradually becoming drier until at the end the tubers were considerably dried out. Compared to the preceding test the amount of rotting was very slight, and its inhibition may be directly attributed to the dryness of the air. The slight amount of rot around the point of inoculation would indicate that the fungus progressed a short distance into the tuber at the beginning of the experiment when the humidity was high but was unable to advance further under the drier conditions. This would indicate that the rotting of tubers already started could be checked by submitting the tubers to lower humidities.

EXPERIMENT 3, APRIL 26, 1916.—A further test on the relation of humidity of the atmosphere to the rot of the tuber was started. The inoculations were made as in the previous experiment, and the same variety of potatoes was used.

Set No. 1. The inoculated tubers were placed in sterile chambers, and moist filter paper was placed in the chambers at the start of the experiment to produce a favorable humidity for the initial penetration of the tuber.

Set No. 2. The tubers were placed in moist chambers in which the atmosphere was kept saturated throughout the experiment.

Both sets were kept at a temperature of 25° C. The results were taken after seven weeks. (Table I.)

TABLE I.—*Comparative amount of rot produced by Fusarium spp. under different conditions of relative humidity*

| Strain. | Set No. 1. | Set No. 2. |
|---|---|---|
| *F. oxysporum* No. 1 | One-third rotted | Entirely rotted. |
| *F. oxysporum* No. 8 | 2-mm. rot | Do. |
| *F. hyperoxysporum* | One-third rotted | Half rotted |
| Control | Healthy | Healthy. |

In all cases where rotting was present the starch grains were not corroded. Culture No. 8 seemed to have a much slower initial growth than the others, thus showing a greater difference between the two sets. In general, it can be clearly seen that the rotting was much greater in set No. 2, where the atmosphere was saturated throughout the experiment. Although the organisms were capable of starting a rot under the moist conditions at the start of the experiment in set No. 1, they were later considerably checked under the drier conditions.

EXPERIMENT 4, MARCH 15, 1917.—Further infection experiments were started under conditions in which the relative humidity of the atmosphere was controlled by the use of various concentrations of sulphuric acid. Previous experiments conducted at the Michigan Agricultural Experiment Station in 1915 and described under experiment 5, in which the relative humidities were carefully controlled, produced very good results with *Fusarium oxysporum*.

The apparatus used in experiment 5 was not available in 1917, so the relative humidities used in experiments 4 and 4A were determined from the tables given by Stevens (*23*). One-quart Mason jars were used, in which were hung small wire baskets containing the tubers, the acid being placed in the bottom of the jar. Tubers of the Rural New Yorker variety were inoculated as in the preceding experiments. They were then placed in the baskets in the sterilized jars and were sealed with paraffin and placed at the desired temperatures. The experiment was run in duplicate. Three strains of *Fusarium oxysporum*, two of *F. trichothecioides*, and one of *F. radicicola* were used for the inoculations. The temperatures used were 5°, 9°, 16°, and 25° C. While these temperatures varied somewhat during the experiment, the extremes did not in any case overlap. The relative humidities obtained by using sulphuric acid remained fairly constant throughout the experiment. One hundred cc. of each of the acid solutions were used for each jar. At the close of the experiment the specific gravity of the solutions was taken, and the calculated humidity at this time was compared with that at the start, with the result that the one having 1.5 per cent relative humidity had changed to 3.6 per cent, the 33 per cent to 49 per cent, and the 66.5 per cent to 74 per cent. These variations were not considered great enough to cause conflicting results.

The results were taken after seven weeks, and the penetration of the tubers was measured in millimeters, as shown in Table II. The number of individuals was so small that slight discrepancies in the tabulated results are found. *Fusarium trichothecioides* produced a slight rot at lower temperatures than *F. oxysporum* but did not produce as extensive a rot at the higher temperatures. At 5° C. the only rotting found was with one strain of *F. trichothecioides*, at 100 per cent humidity. No rotting was found at the temperature of 9° at the lower humidities, but there was slight rotting at this temperature at the higher humidities, especially with *F. trichothecioides*. It is noticeable that at 9° with the relative humidities of 66 and 100 per cent, the amount of rotting is greater than at the increased temperature of 16°, with the relative humidities of 1 and 33 per cent The same comparative results are found between the amount of rotting taking place under the several humidities at a temperature of 16° and of 25°. The results do not conform with the report of Jamieson and Wollenweber (*8*), that penetration of the tuber by *F. trichothecioides* is favored by low humidities. The work by Link (*9*) and Wilcox, Link, and Poole (*26*), however, would indicate that more rapid rotting takes place in an atmosphere of high humidity, thus agreeing with the results shown in this experiment. The results of *F. oxysporum* accord well with those obtained in experiment 3.

TABLE II.—*Extent of penetration of tubers in experiment 4*

| Temperature | Approximate relative humidity | *Fusarium oxysporum.* | | | *Fusarium trichothecioides.* | | *Fusarium radicicola*, Strain 29. |
|---|---|---|---|---|---|---|---|
| | | Strain 1. | Strain 8. | Strain 32 | Strain 28. | Strain 31 | |
| °C. | Per cent. | | | | | | |
| 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 33 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 66 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 100 | 0 | 0 | 0 | 0 | 3 mm. | 0 |
| 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 33 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 66 | 0 | 1 mm. | 0 | 0 | 1 mm. | 0 |
| | 100 | 0 | 1 mm. | 0 | 1 mm. | 3 mm. | 1 mm. |
| 16 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 33 | 1 mm. | 0 | 0 | 0 | 0 | 0 |
| | 66 | 2 mm. | 3 mm. | 0 | 1 mm. | 1 mm. | 0 |
| | 100 | 10 mm. | 10 mm. | 0 | 1 mm. | 1 mm. | 2 mm. |
| 25 | 1 | 5 mm. | 10 mm. | 1 mm. | 0 | 0 | 1 mm. |
| | 33 | 10 mm. | 15 mm. | 3 mm. | 0 | 2 mm. | 6 mm. |
| | 66 | 20 mm. | ½ rot. | ½ rot. | 1 mm. | 25 mm. | 6 mm. |
| | 100 | ½ rot. | Complete rot. | ¾ rot. | 1 mm. | ½ rot. | Complete rot. |

EXPERIMENT 4 A, MAY 16, 1917.—In order to check up the possible error due to differences in the age of the tubers used in the various tests, the following experiment was started. New tubers of the Bliss Triumph variety were used in comparison with tubers of the same variety that had

been kept in cold storage from the previous year. The experiment was conducted in the same way as experiment 4, and the same cultures of *Fusarium oxysporum*, *F. trichothecioides*, and *F. radicicola* were used for inoculations. Only two temperatures were used, 13.5° and 25° C., as well as two humidities, 33 and 100 per cent at each temperature. The results shown in Table III were taken after six weeks.

As in experiment 4 the rotting was much greater at the high temperatures and the high humidities. At the lower temperature of 13.5° C. there was no distinct difference between the amount of rotting in the old and new tubers, due to the very slight penetration at this temperature. In the old tubers at 25° the infection in every case had been rapid and the rotting had progressed much further than in the new tubers. These results support the statement of Bisby (3) that old tubers are more susceptible to rot than new tubers

TABLE III.—*Extent of penetration of old and new tubers*

| Temperature. | Approximate relative humidity. | Tubers. | *Fusarium oxysporum* | | | *Fusarium trichothecioides.* | | *Fusarium radicicola*, Strain 29. |
|---|---|---|---|---|---|---|---|---|
| | | | Strain 1 | Strain 8. | Strain 32 | Strain 28. | Strain 31. | |
| *°C.* | *Per cent.* | | | | | | | |
| 13.5 | 33 | New. | 2 mm. | 5 mm. | 1 mm. | 4 mm. | 2 mm. | 5 mm. |
| | | Old. | 2 mm. | 2 mm. | 1 mm. | 7 mm. | 10 mm. | 5 mm. |
| | 100 | New. | 5 mm. | 5 mm. | 1 mm. | 2 mm. | 2 mm. | 5 mm. |
| | | Old. | 5 mm. | 4 mm. | 1 mm. | 5 mm. | 6 mm. | 5 mm. |
| 25 | 33 | New. | 20 mm. | 2 mm. | 3 mm. | 4 mm. | 5 mm. | ¼ rot. |
| | | Old. | Contaminated. | 15 mm. | 15 mm. | ¼ rot. | 5 mm. | ¼ rot. |
| | 100 | New. | Contaminated. | ¼ rot. | Contaminated. | 2 mm. | 2 mm. | ¼ rot. |
| | | Old. | 5 mm. | ½ rot. | 15 mm. | 10 mm. | ½ rot. | Complete rot. |

EXPERIMENT 5, 1915.—The results of earlier experiments having indicated that the influence of the relative humidity was nearly as great as that of temperature, it was decided to run a more complete test on the effect of the relative humidities at different temperatures. Since no apparatus was available by which the relative humidity and temperature could be controlled at will, it was necessary to construct one.

The principle employed in experiment 4 of using sulphuric-acid solutions of varying specific gravity in a closed chamber to obtain the different relative humidities was not used in this test. In preliminary experiments conducted in the same way as experiments 4 and 4A, the infection usually resulted in a softrot which gave good comparative results, but the type of rotting was not similar to that usually found in storage. Cultures from these softrots invariably yielded the *Fusarium* sp. used in the inoculation, and no bacteria were present in any case. Apparently

the absence of any aeration was the cause of this abnormal type of rotting, and the following method was devised to allow for aeration.

The principle finally decided upon was that of passing a current of air, kept at a constant pressure, through sulphuric-acid towers and then over calcium chlorid and sodium hydrate. This gave a constant stream of dry, sterile air. The air was then passed over sterile water to bring it to a desired humidity. The amount of water necessary for a given humidity was determined by trials, and the air was then passed into the jars containing the tubers. An outlet was provided at the bottom of the jar. These jars were connected separately with the current of air and not in series. Relative humidities were obtained and used throughout the experiment as follows: 1, 30, 70, and 100 per cent. These relative humidities were used at three different temperatures—9°, 12.5°, and 25° C. The set at 25° was placed in an incubator in the laboratory, the set at 12.5° was placed in a special low temperature incubator, and the set at 9° was placed in a well-insulated ice box. Each of these temperatures was maintained within a variation of 2° throughout the experiment. In this way four gradations of humidity at each of three temperatures were obtained. The method provided the tubers with sufficient aeration and secured sterile conditions throughout the experiment, since the jars containing the tubers were not moved or opened until the end of the period.

The humidity readings were taken by the wet- and dry-bulb method, the thermometers being inserted into the stream of air at the entrance to the jar. The readings were found to vary, and at least 10 trial readings were taken for each jar after the preliminary determinations were made and the apparatus was set up. These readings ranged as follows: 1 to 10 per cent, 20 to 40 per cent, 60 to 80 per cent, and 90 to 100 per cent. These were the greatest extremes found; and since a knowledge of the approximate relative humidity is all that is necessary in an experiment of this kind, these readings were taken to be sufficient, inasmuch as they showed a gradual gradation from approximate dryness to saturation. The ranges given above simply denote the possible error due to the method of taking the readings. The humidity necessarily remained constant, since the temperature, water surface, and air pressure were constant. It was found to be impossible to use the wet- and dry-bulb method to determine the relative humidity at the lower temperatures. The changes of temperature caused by opening the door to make the determinations were found to change the readings. Therefore the sets at 9° and 12.5° C. were installed temporarily at 25°, the preliminary determinations were made, and the readings were taken at that temperature and corrections made by the use of psychometric tables (*13*).

The large battery jars were fitted with wire screen supports, and six tubers were used in each jar—four inoculated and two controls. The

control tubers were separated from the inoculated ones by a thin layer of cotton. The entire apparatus was disinfected with formaldehyde gas before the experiment was set up.

The tubers used were of the Up-to-Date variety. They had been kept over winter in a cool cellar, and a few sprouts which had started were removed. They were inoculated by wounding the epidermis and placing several drops of spore suspension in the wound. They were then placed in the jars which were closed with cork tops and paraffined. The inoculations were made with *Fusarium oxysporum* No. 8. The jars were opened up and the tubers examined after five weeks.

Set 1 (9° C.). At 10 and 30 per cent humidity the tubers were all healthy.

At 70 per cent the tubers were sound with no penetration, although there was a slight growth of mycelium on the surface of the tuber at the point of inoculation.

At 100 per cent the condition of tubers was the same as at 70 per cent, except that the external growth of mycelium was greater. All the control tubers of this set remained healthy, and both the controls and inoculated tubers had sprouted.

Set 2 (12.5° C.). At 10 per cent humidity the tubers were healthy. There was no invasion of the tissues.

At 30 per cent, same as above with a slight external growth of mycelium at the point of inoculation.

At 70 per cent the tubers were about the same as at 30 per cent. (Pl. 10, A.)

At 100 per cent invasion of the tissue had taken place for about 2 mm. beyond the wound, causing a slight browning of the tissue. On the surface there was a slight brown discoloration for several millimeters surrounding the point of inoculation and a slight growth of aerial mycelium. All the control tubers in set 2 remained healthy, and both control and inoculated tubers were sprouting normally. (Pl. 10, B.)

Set 3 (25° C.). The control tubers remained healthy and sprouted at 10 and 30 per cent relative humidity, while at 70 and 100 per cent there was a slight disorganization of the tissue around the eyes and the sprouts were all dead. No actual rotting was present or any fungus growth.

With infected tubers at 10 per cent humidity all inoculations were successful and uniform. The fungus invaded the tissue for 2 cm. around the wound. Immediately below the surface at the point of inoculation there was in every case a cavity lined with a white mycelial growth. The tissne surrounding the cavity was of a granular appearance. Examined under the mieroscope it appeared to be made up of a tangled mass of mycelium and starch grains. A softrot extended out from this area, the tissue being light brown in color and completely invaded by mycelium (Pl. 10, C).

At 30 per cent humidity the rotting took place in the same manner as at 10 per cent, except that the cavity was larger and the rot extended through about 50 per cent of the tuber (Pl. 11, B).

At 100 per cent humidity there was a total rot of all tubers, most of the surface being covered with a white mycelial growth (Pl. 11, C).

The results of this experiment show very clearly that relative humidity plays a very important part in determining the amount of rot produced by *Fusarium oxysporum*. The only rot appearing at the low temperature of 12.5° C. was in an atmosphere of 100 per cent humidity. Even at the high temperature of 25° complete rotting did not take place at the lower humidities. A gradual increase in the amount of rot corresponding to the increase in humidity was present in every case. The fungus can live and sporulate at the lower temperatures and lower humidities used in this experiment but apparently is not capable of penetrating the tubers under these conditions. It can be safely concluded that *F. oxysporum* under good storage conditions is not capable of producing a tuber-rot of great importance.

## CONCLUSIONS

(1) *Fusarium oxysporum*, *F. trichochecioides*, and *F. radicicola* are all capable of producing a rot of the potato tuber.

(2) In pure culture the amount of growth of all three species is nearly equal at 25° C., *Fusarium oxysporum* and *F. radicicola* increasing in growth up to 30°, where they produce their maximum growth. The growth of *F. trichochecioides* decreases above 25°, until at 30° very little or no growth takes place. It is more tolerant of the lower temperatures than the other two species.

(3) Preliminary tests with different liquid media would indicate that the cardinal points for growth of these Fusaria vary to some extent with the medium used.

(4) Experimental infection of tubers was produced with all three organisms under various conditions of temperature and relative humidity.

(5) Preliminary tests with *Fusarium oxysporum* indicated that the relative humidity plays a very important part in determining the amount of rotting.

(6) In comparative tests with new and old tubers there is a distinct difference in the amount of rotting under the same conditions. The rotting was much more rapid and progressed much further in the old than in the new tubers.

(7) Comparative tests with all three species at controlled relative humidities from 1 to 100 per cent and at controlled temperatures from 5° to 25° C. proved conclusively that—

(*a*) A temperature of 25° C. is favorable for the production of a tuber rot by *Fusarium oxysporum*, *F. radicicola*, and *F. trichothecioides*.

(*b*) *Fusarium oxysporum* grows more rapidly and produces a more extensive rotting of the tuber than the other two at a temperature of 16° C. and above.

(*c*) *Fusarium trichothecioides* is capable of producing a rot at much lower temperatures than the others, in some cases causing rotting at 5° C.

(*d*) The relative humidity plays a very important rôle in determining the progress of tuber rots and has the same influence on all three species. In every experiment it was noticeable that there was a gradual increase in the amount of rot corresponding to an increase in relative humidity. With a high humidity at a given temperature the rotting was always greater than at a temperature 5° to 10° C. higher but with a low humidity. The Fusaria used can all live and sporulate at the low temperature of 9°, and with low relative humidities, but they are not capable of producing a rot under these conditions.

Inasmuch as the three species of Fusaria used in these experiments represent the common types causing storage-rots of potatoes, it is clear that considerable attention should be given to moisture as well as temperatures where incipient rot occurs in stored tubers. It is also entirely probable that a rotting of the tubers initiated at high temperatures and high relative humidities could be completely checked by submitting the tubers to lower temperatures and lower humidities.

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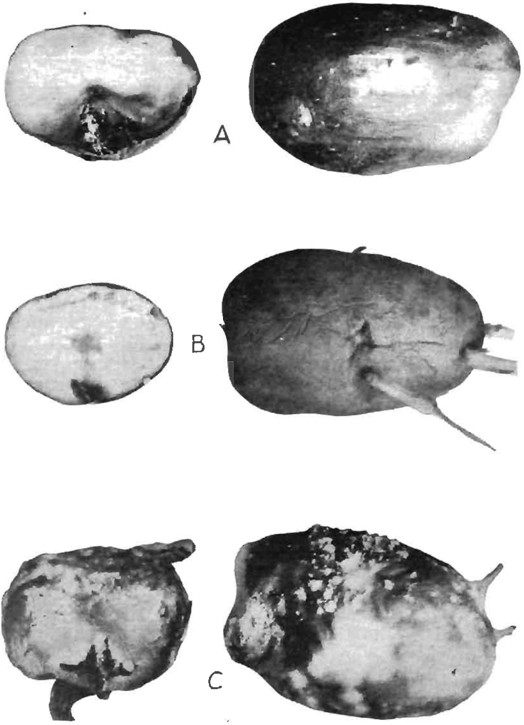
PLATE 10

Tubers inoculated with *Fusarium oxysporum* and kept for five weeks at the following temperatures and humidities:

A.—12.5° C., 70 per cent relative humidity.
B.—12.5° C., 100 per cent relative humidity.
C.—25° C., 10 per cent relative humidity.


A
B
C

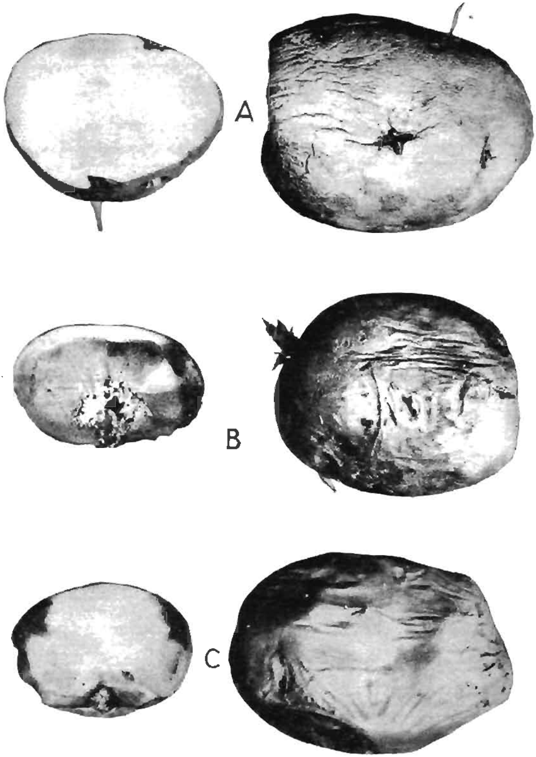

A
B
C

PLATE 11

Tubers inoculated with *Fusarium oxysporum* and kept for five weeks at the following temperatures and humidities:

A.—25° C., 30 per cent relative humidity.

B.—25° C., 70 per cent relative humidity.

C.—25° C., 100 per cent relative humidity.

# BLACKLEG POTATO TUBER-ROT UNDER IRRIGATION

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## OCCURRENCE AND GENERAL APPEARANCE

A bacterial field decay of the potato tuber, the real nature of which has not heretofore been adequately explained, prevails in certain irrigated sections of the West. In early harvest, when the diseased tubers are apparently free from fungous invasion, the trouble has sometimes been assumed to be "sunscald"; during the winter months it has frequently been taken for a form of freezing injury. In other instances it has been confused with the so-called "jelly-end rot" and attributed either to *Fusarium radicicola* Wollenw. or to *F. oxysporum* Schlecht. It is probable, also, that on superficial examination some such material has been classed as "leak" (*Pythium debaryanum* Hesse), when conditions favored an extremely rapid progress of the decay, whether in the field or in transit.

Specimens of this decay were received by the writers in 1917 and 1918 from Idaho, Nevada, and California. In material received in August the decay was soft and mushy (Pl. 12, A–C). The affected tissues were in part brown to black, but mostly only slightly colored or colorless, though with a darker margin on the border line between the healthy and diseased portions. Disintegration, originating at one end of the tuber, was advancing irregularly over the surface. In some areas the decay was confined to the outer layer, just beneath the epidermis, while in others the deeper tissues also were involved. As a rule, the disease started at the stem end, but occasionally the eye end became infected first (Pl. 12, B). Decaying material usually possessed a disagreeable odor. It is this soft type of the rot which some were inclined to regard as sunscald injury.

Specimens received later in the season, during the months of November and December, presented an entirely different appearance. The affected portions were not mushy, but more or less tough or dry and shrunken (Pl. 12, D). The diseased area was dark brown in color, except when a fresh decay developed under favorable conditions deeper in the tissues. In the latter case it was practically of the same color as the normal flesh of the tuber, but soft and mushy in consistency. When such tubers were cut open and the cut surfaces exposed to the air, the diseased portions turned brown or even black. If the progress of the decay is completely arrested, the trouble may readily be mistaken for

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an inactive stage of jelly-end rot or for an after effect of freezing injury, particularly if the disease has made but little headway. The true nature of such obscure cases of the disease may be revealed with certainty only by a series of cultural studies, coupled with experimental work and field observations.

## CAUSAL ORGANISM

Isolations were made from every tuber of each of the four samples received from the West in 1917–18. The results were surprising. In no case was *Fusarium radicicola* obtained; only one tuber yielded *F. trichothecioides* Wollenw. (from Nevada), two yielded Rhizoctonia (from Nevada), two *F. oxysporum* (from California), and a few gave miscellaneous, apparently saprophytic, fungi. Bacteria, on the other hand, were constantly present in the cultures, even when slightly acidulated potato agar was used. Carpenter (*2*)[1] noted the presence of bacteria in jelly-end rot material, but he regarded these organisms as saprophytic, as they probably were. In the writers' cultures, however, the constant prevalence of one type of bacterial colony in the dilution plates was significant and warranted a detailed study of this organism. In the subsequent inoculation experiments with pure cultures it proved to be strongly pathogenic and produced a progressive decay of the tubers as well as a disease of the stems. A study of the cultural and biochemical features of the organism showed them to be fully in accord with the published description of the blackleg bacillus (*5*).

### MORPHOLOGY

Short rod with rounded ends, also short chains; 0.5 to 0.9 × 1.0 to 2.2 μ, average 0.6 × 1.8 μ; flagella few, peritrichiate; no endospores and no capsules; stains well in aqueous gentian violet, aqueous methylene blue, aqueous fuchsin, anilin water gentian violet, alkaline methylene blue, and carbol fuchsin.

### CULTURAL FEATURES

AGAR STROKE.—Growth moderate, filiform, flat to slightly raised, glistening, smooth, slightly opalescent; white, no odor; consistency slimy to butyrous; one strain distinctly viscid at first, but after a few replatings it lost its viscidity.

POTATO.—Growth moderate to abundant; filiform at first then spreading, slightly convex changing to flat, glistening, smooth to slightly rugose, yellowish white or dirty white; a decided odor of decayed potatoes on the third to fourth day at 22° to 25° C.; consistency somewhat slimy; medium slightly grayed at first, changing later to either plainly gray, or purplish, or brown, or a combination of these shades.

[1] Reference is made by number (italic) to "Literature cited," p. 91–92.

AGAR STAB.—Growth somewhat best at top, abundant, spreading, filiform to slightly papillate.

GELATIN STAB.—Growth best at top, filiform along the line of puncture, liquefaction, beginning on the first day at 20° C., varying in shape from crateriform or funnel-shaped to saccate and broadly infundibuliform, complete in 7 to 12 days.

NUTRIENT BROTH.—Usually slight ring and slight granular pellicle in young cultures, clouding moderate to strong, persistent; medium not discolored, odor absent; sediment compact, granular, somewhat dirty white; one strain decidedly viscid at first, but losing this character after a few replatings.

MILK.—Coagulation and extrusion of whey at 25° C., beginning on the fourth day; coagulum not digested; one strain extremely viscid at first, but losing its viscidity in later replatings; medium not discolored.

ACID PRODUCTION IN MILK.—A slight increase of acidity in milk cultures was noticeable after 24 hours. Two series of tests were made at certain intervals within the period of 20 days, two to three cultures being used on each day for every strain. The average progress of the acidity of three western strains was as follows:

| AGE OF CULTURE. | REACTION IN FULLER'S SCALE. |
|---|---|
| 1 day | +12.28 |
| 2 days | +13.49 |
| 3 days | +22.13 |
| 5 days | +28.00 |
| 10 days | +32.90 |
| 20 days | +41.12 |

The average reaction of the control tubes was +11.8 Fuller's scale. Two strains received from Dr. W. J. Morse, of the Maine Agricultural Experiment Station, were tested along with the western strains and gave similar reactions, one ("B. sol.") showing 37.0 and the other ("IIIA") 40.75 acidity on the twentieth day. The cultures were grown at 22° to 25° C.

LITMUS MILK.—At 22° to 25° C. bleaching was complete at the end of three weeks; thorough reddening was accomplished in seven weeks.

GELATIN COLONIES.—Growth rapid, form round, edge entire, liquefaction saucer-shaped.

AGAR COLONIES.—Surface colonies; growth rapid, usually round, but occasionally somewhat irregular, flat to slightly raised, entire to slightly undulate, finely granular with an internal ring surrounded by radiate striations; color pearly white, bluish opalescent by transmitted light; maximum diameter of colonies after 2 days 2 mm., after 3 days 4 mm., after 7 days 7 mm., after 14 days 9.5 mm. Buried colonies lens-shaped to nearly spherical, edge entire, color slightly yellow under hand lens.

FERMI'S SOLUTION.—Moderate clouding in 2-day cultures; later growth becomes copious.

54818°—21——3

COHN'S SOLUTION.—No growth.

USCHINSKY'S SOLUTION.—Growth was somewhat irregular in the ordinary Uschinsky's solution but was uniform and copious in the modified Uschinsky's solution, clouding being very strong on the fifth day.

SODIUM CHLORID IN BOUILLON.—Growth slightly inhibited by 3 per cent and more so by 4 per cent; no growth appeared in 5 per cent tubes until the third day, and only occasional tubes containing 6 per cent were clouded after 5 days. Morse reports no clouding for *Bacillus atrosepticus* Van Hall in concentrations higher than 5 per cent. At the end of two months, when conditions remained unchanged, transfers were made from 6 per cent and 7 per cent sodium-chlorid cultures of the western strains to sterile broth. In 48 hours all the transfers from 6 per cent solutions showed growth, and in three days clouding appeared in the majority of the transfers from the 7 per cent solutions, the remainder being dead.

GROWTH IN BOUILLON OVER CHLOROFORM.—Growth somewhat restrained at first, but increasing gradually. On the fourth day there was a strong and uniform clouding in all cultures.

BEST MEDIUM FOR LONG-CONTINUED GROWTH.—Morse considers that in the case of *Bacillus atrosepticus* neutral beef bouillon is best for this purpose. In the western strains the writers observed that the organisms can live even longer on the agar than on the broth when grown at ordinary laboratory temperature of 22° to 25° C. Their death on agar appears to be primarily associated with drying of the medium, while in broth it seems to be due to certain chemical changes in the substratum and takes place sometime before the liquid dries up completely. Six series of parallel broth and agar cultures were made and tested at different intervals, from 8 to 36 weeks, by making transfers to tubes of sterile broth. It was found that occasional broth cultures showed a somewhat weakened vitality, as demonstrated by retarded clouding, at the age of 16 weeks; some died after the expiration of 20 weeks, and none lived beyond 26 weeks. On the other hand, in no case was the agar culture dead before 26 weeks, and some remained alive even after 36 weeks. The experiment was carried on with 10 cc. of medium in each test tube.

## PHYSICAL AND BIOCHEMICAL FEATURES

FERMENTATION TUBES.—Gas and acid production as well as growth in the closed arm was observed with dextrose, lactose, and saccharose. No acid and no gas with glycerin in cultures 1, 3, and 5 days old.

AMMONIA PRODUCTION.—Feeble (tested by Folin's aspiration method).

NITRATES IN NITRATE BROTH reduced to nitrites.

INDOL PRODUCTION.—Positive, but very feeble both in young and old cultures.

TOLERATION OF HYDROCHLORIC ACID AND SODIUM HYDRATE.—The writers' western organism grew in tubes having an initial reaction before

final sterilization of +20 and −20, Fuller's scale, but not in those adjusted to +30 or to −30. Uninoculated tubes held as controls and titrated at the close of a 24-day incubation period showed marked changes from the original reaction, due doubtless in considerable measure to the absorption of gases with resulting chemical change. Tubes calculated for an initial reaction of +30 showed a final reaction of from +20 to +25; those originally +20 were about +15; those −30 were about −10; and those −20 were about −6. Transfers from inoculated tubes calculated for an initial reaction of +30 and above and −30 and below made 24 days after inoculation developed growth in some cases in tubes from +30, but not in those from more acid reactions nor from the alkaline broths.

VITALITY ON CULTURE MEDIA.—Long on bouillon, but still longer on agar.

TEMPERATURE RELATIONS.—In freshly inoculated broth cultures exposed 10 minutes, occasional retardation of clouding began at 45° C.; occasional growth was noted at various points between 46° and 50°; and in no case was growth present after heating above 50°. Optimum temperature for growth about 25°. Maximum temperature for growth between 33° and 35°. Minimum temperature for growth below 5°.

EFFECT OF SUNLIGHT.—Thinly sown agar plates exposed on ice for 30 minutes the latter part of March in Washington, D. C., resulted in 100 per cent killed.

CYTASE PRODUCTION.—Five-day-old 30-cc. broth cultures in 300-cc. Erlenmeyer flasks were precipitated by 160 cc. of 80 per cent alcohol, filtered, and the precipitate dried promptly in the air. The papers containing the dried precipitate were washed with 30 cc. of water, and the washings were received in a flask to which a few drops of toluene and three raw Irish potato disks 15 by 2 mm. were added. The disks gradually assumed a soft, cheesy consistency but did not entirely disintegrate. Microscopic examination showed the cells had lost coherence through softening of the middle lamella. The cellulose lamella and the starch content of the cells showed no evidence of change. Controls with uninoculated broth did not soften the disks.

GROUP NUMBER 221.1113033

The last three points in this group number differ from those given by Morse (*5*) but coincide with the respective figures in Jennison's (*3*) revision, as reported by him at the fourth annual meeting of the Pacific Division of the American Phytopathological Society. The writers feel, however, that this may be largely a matter of interpretation of certain results and not necessarily an indication of actual difference in the organisms. Jennison studied 12 different strains of the blackleg bacillus, including several of Morse's strains; but the results he obtained, apparently,

were identical for all strains. The writers regard their western strains as nonchromogenic, although a certain yellow discoloration in cooked potato cultures might be taken as a suggestion of yellow pigment. They obtained no evidence of diastatic action on potato starch, nor of acid production with glycerin.

It may, therefore, be concluded on the basis of the characters described that the pathogenic bacillus isolated by the writers from a peculiar soft decay of western potato tubers is essentially identical with the organism causing the blackleg disease of potatoes for which Appel's binomial *Bacillus phytophthorus* is regarded to be correct by Smith (7). Morse (5), who was unable to obtain an authentic culture of Appel's strain for his comparative studies of various blackleg organisms, believed that *B. atrosepticus* should be chosen in preference to other names he had under consideration, but stated that—

There is nothing in the data here presented which bears on the relation between the organism originally described by Dr. Appel (*1*) as *B. phytophthorus* and the other strains of blackleg bacteria.

## EXPERIMENTAL WORK

The pathogenicity of the bacterial organism described above was established by means of the following laboratory, greenhouse, and field experiments.

### PLANTING OF THE ORIGINAL MATERIAL

Preliminary to the inoculation work some of the diseased western material was planted in the greenhouse as soon as the isolations were completed. Four tubers were selected and cut in halves so as to make eight seed pieces. Each piece was planted in a separate large pot filled with sterilized soil. Of these seed pieces one decayed completely in the soil before germination, six produced diseased plants, and one produced a plant considerably weakened though not clearly diseased. Some of the affected plants decayed while very young, others grew up to practically normal size, developing blackening of the stem above ground and brown to black lesions on the underground portions. The lower leaves turned yellow, but the upper leaves wilted while green. In two cases the blackening of the stems was very intense (Pl. 13, A) while in the remainder the appearance was less typical of the familiar field symptoms of the disease as it occurs in the eastern sections of the country. Blackening of the pith of the stem developed to the very top in one case. When an affected plant was removed from the pot and the soil was carefully washed off, it could be seen that the infection had spread from the seed piece to the stem (Pl. 13, B). No tubers were produced in this experiment. Healthy sprouting Irish Cobbler tubers were replanted in these pots, but no infection was contracted by this new set of plants.

## INOCULATION OF HEALTHY TUBERS IN THE LABORATORY

Over 60 tubers both new and old of the Netted Gem as well as of the Irish Cobbler varieties were inoculated in small lots at different times with three strains of the western decay bacillus. When inoculations were made in wounds of any kind, whether on the side of the tuber or at either end of it, the results were invariably positive. The progress of the decay was much slower when uninjured potatoes were inoculated. In these latter cases the organism penetrated either through the eyes or through the young growing sprouts. If the infected potatoes are removed from the moist chamber after the decay has made considerable headway and are exposed to the dry air of the laboratory, the diseased tissues become shriveled and folded, resembling very closely the original specimens of natural infection (Pl. 14, A, C). Ordinarily if the infected material is kept in moist chambers the decay is soft, mushy, spreading either equally throughout the tissue or sometimes more on the surface of the tubers, and is not confined to their piths as is usual in typical cases of blackleg. The color of the decaying areas ranges from that of the normal flesh to light or dark brown, often with blackish streaks or stripes in younger portions nearer to healthy tissues, but never black throughout. The margin is usually well defined, and there is no gradual transition from dead to sound tissues. This internal appearance changes considerably when tubers are taken from the moist chamber and are exposed to drying. The decay of the bark is then more or less arrested, and the disintegration centers mainly in the pith, so that a more or less sound shell surrounds the centers of the active decay. The diseased tissue is brown to black, the older regions becoming slimy (Pl. 14, B, D). In all cases the decay gives off a very strong putrefactive odor.

## PLANTING OF ARTIFICIALLY INOCULATED TUBERS IN THE GREENHOUSE

Seven Irish Cobbler tubers inoculated with the western bacterial organisms and partly decayed were planted in sterilized soil in pots. Four showed subsequently a stem decay and three remained apparently unaffected. One plant became girdled and died early. The disease appeared first on the remaining three plants in the form of black streaks in various positions on the stems, particularly at the leaf petioles. Later on in some instances the entire stalk became black at the base. Tuber-rot did not appear except on one tuber in one of the diseased pots. In this case it was a soft, watery decay, light in color, not typical for blackleg. The causal organism, identical with the original strains, was, however, recovered from this area. Healthy sprouting Irish Cobbler tubers were immediately planted in the same pots in which these specimens were grown. The new plants were very vigorous, and none of them contracted the disease.

INOCULATION OF HEALTHY STEMS IN THE GREENHOUSE

The stems of four young healthy potato plants were inoculated with 24-hour-old broth cultures of the three western strains of the bacillus injected by means of a hypodermic needle. A severe decay with an accompanying blackening resulted in all cases.

FIELD EXPERIMENTS

These experiments were conducted for two successive years at Arlington Farm, Va. In 1919 Netted Gems and Irish Cobblers were used. The tubers were inoculated with the western strains of the blackleg organism a few days before planting. Six whole tubers and 20 halves of the first variety and 9 whole and 19 halves of the second variety were planted. In addition a number of uninoculated pieces of each variety were planted for controls. Planting was done on May 5. One half-tuber seed piece of each variety decayed in the ground. On July 1 one hill from the cut seed of Irish Cobblers was noted to show secondary symptoms characteristic of blackleg—namely, yellowing and rolling of the leaves. There was no blackening of the stem above ground. The underground portions, however, showed brown lesions and a brown rot of the stem at the point of attachment to the seed piece and somewhat above it. None of the remaining plants showed symptoms of the disease. At digging time, on September 15, no decay of the tubers was found, with the exception of one very small tuber of the Netted Gem variety which showed a soft bacterial decay at the stem end. The progress of the decay, however, was checked, and the affected portion fell off, leaving only the sound part, so that the recovery of the causal organism was not possible.

Since the hot weather after May 5 might have had something to do with the slight progress of the disease in 1919, two sets of plantings were made on another piece of ground on the same farm in 1920—one on April 8 and the other on May 6. Only Irish Cobblers were used this time. Twelve tubers were cut in halves through the inoculated wound so as to make 24 seed pieces for each of the two series. Inoculations were made a few days before planting. Eight tubers were inoculated with the three western strains of the blackleg organism and 4 with the "B. sol." strain received from Dr. Morse. Up to July 20 four hills out of 16 inoculated with the western strains in the earlier planting and 2 out of 8 inoculated with "B. sol." in the same series showed typical field symptoms of blackleg, including an intense blackening of the base of the stem. On the other hand, no hill of the series inoculated with the same organisms and on the same plan, but planted one month later, showed any signs of infection. At harvesting time, on July 20, a number of tubers in the planting of April 8 showed blackleg-rot, and in the later planting only 2 tubers were found showing the same decay. It appears, therefore, that the earlier planting, when the soil and the air temperatures were

lower and the soil moisture was more abundant, greatly facilitated the development of blackleg.

## FIELD OBSERVATIONS

The typical case of blackleg-rot on round varieties in the East has been figured in publications and charts issued by several agricultural institutions. As a rule, decay begins at the stolon end of the tuber with a comparatively small amount of rot visible on the outside or often only a small, black, circular opening. This opening leads to the interior of the tuber, where a progressive decay develops in the form of an irregular black, soft, or slimy hollow until nearly all of the tuber is consumed (Pl. 15, D, E). However, the development of the disease may deviate from this type even in eastern and northern sections of the United States, when conditions are abnormal and favorable to the disease, such as those in moist places or in wet seasons. Morse stated (*4*) with reference to blackleg in Maine that—

> When this disease occurs on a field it doubtless is responsible for much of the soft rot of the tubers observed in wet seasons.

It appears from certain observations made by the writers that under conditions of excessive soil moisture the bacteria in stems or seed tubers may be carried at least to the adjoining tubers of the same hill. The latter then become infected from the outside, or, if they are already infected through the stolons, the infection spreads in moist surroundings more rapidly on the outside over the surface of the tuber, or evenly throughout the flesh. Specimens of this sort were observed on the Eastern Shore of Maryland and Virginia (Pl. 15, G) and in Wisconsin, Minnesota, and Washington (Pl. 15, F) on various round types of potatoes. More accentuated symptoms of this order were found in irrigated sections of Colorado. The most peculiar manifestations of the blackleg tuber-rot were seen in the Snake River Valley of Idaho, where the Netted Gem variety is grown on a large scale. The following forms were observed there during a field survey arranged by the Office of Cotton, Truck, and Forage Crop Disease Investigations in 1920.

1. The stem-end rot of pointed-end Netted Gems. The external appearance of this form is extremely misleading (Pl. A, 1–4). It becomes prevalent in southeastern and eastern Idaho during the latter part of the season, shortly before the harvest. The relatively low temperature prevailing at this time of year is, no doubt, an important factor in the rapid progress of the disease. If the soil has plenty of moisture, freshly dug affected tubers show no shrinkage and preserve their natural shape (Pl. 15, A–C.). In the course of two weeks the shrinkage is evident and the decay takes on an inward trend (Pl. A, 4). By another two weeks, drying and folding of the decayed tissues become very pronounced, and the external appearance at this stage of the decay may well pass as an

illustration of any of a number of stem-end tuber-rots (Pl. 16, A, B). When a Netted Gem tuber of pointed shape affected with this type of the disease is cut open longitudinally as soon as it is removed from the ground, four distinct regions of decay may, as the rule, be seen: (*a*) The extreme stem-end region is usually decayed throughout; it is mushy or slimy in consistency and dark brown to black in color; in the field this seldom extends deeper than the outer demarkation line of the decay, but in storage under favorable conditions the disintegration advances more rapidly in the inner tissues of the tuber, taking on a cup-like shape and leading ultimately to the formation of a slimy cavity (Pl. A, 4; 14, B, D; 16, B). (*b*) An area of fresh decay appears within the core just beneath the first region; it is practically colorless, though it occasionally contains dark or black streaks, and in the very early stages it has the consistency of hardened butter (Pl. A, 4). (*c*) The cambium layer shows a brown discoloration extending sometimes close to the eye end; in advanced stages a portion of this region nearest to the stem end is more or less disintegrated and forms a channel attentuating toward the eye end until it gradually transforms into a mere browning of the vascular network which also gradually loses its intensity and finally disappears altogether; this condition is very distinct with some freshly dug tubers, but later on with the inward progress of the decay it becomes less pronounced (Pl. A, 1, 4; 16, B). (*d*) The decay of the outer layer develops in the bark region, is soft but not mushy in consistency and more or less dark-brown in color; it frequently extends over the tuber much farther than the pith decay, but not always as far as the cambium discoloration; its progress is checked after tubers are dug and exposed to drying (Pl. A, 1, 2; 16, B).

2. The shallow stem-end rot of round-shaped tubers. This type was observed mostly on the Idaho Rurals. Under conditions of abundant moisture the bacterial infection spreads from the stem end over the surface of the tuber and penetrates into the bark region, though not very deeply. When such tubers are taken out of the ground and exposed to the sun, as happens at digging, the infected areas dry up very promptly and form hard, black, shallow patches (Pl. 16, E). The condition may easily be mistaken for the black fieldrot described by Pratt (*6*) and attributed to *Fusarium radicicola*. If, however, the tubers are again transferred to a moist place with a moderate temperature, a soft, mushy bacterial decay is likely to develop beneath these dry areas. On the contrary, under conditions unfavorable to the blackleg decay the status may either remain unchanged or become complicated by the entrance of various Fusaria and other rot-producing fungi. In the latter case it is impossible to determine the original cause of the disease.

3. Siderot of either round or long potatoes. It may penetrate inside of the tuber to a considerable depth, and when a freshly dug diseased

tuber is cut open it reveals a colorless buttery or mushy decay with black streaks, usually on the border line of the diseased and healthy tissues. If exposed to drying the decayed areas may become spongy and very much resemble the texture which is usually observed in Fusarium rots (Pl. 16, D). In many instances, however, if the decayed region is sufficiently deep to prevent complete drying, sections through such tubers may show an inner layer of active bacterial decay. As is the case with the other forms of blackleg-rot this form, too, may become further invaded with various rot-producing or saprophytic fungi. The writers had under their observation a tuber of this type with a copious growth of Rhizoctonia on the outside all over the diseased area, while soft bacterial decay was still progressing within the tuber even in the dry laboratory atmosphere (Pl. 16, C).

## SUMMARY

(1) An organism isolated from western stem-end rotting potatoes is identical with *Bacillus phytophthorus* Appel in all the essential characters commonly considered in the determination of bacterial species.

(2) It is pathogenic to the potato, and inoculations of healthy stems or tubers with pure cultures produce, respectively, a rapid, soft decay of stems or a tuber-rot.

(3) Blackleg tuber-rot under the field conditions in certain irrigated sections of the West, particularly in pointed-end Netted Gems, takes on a form atypical of the familiar manifestation of this disease in the East.

(4) The external appearance becomes especially confusing when the affected areas dry up and shrivel in storage, but usually the trouble may be identified by cultural work or by planting diseased tubers under control conditions.

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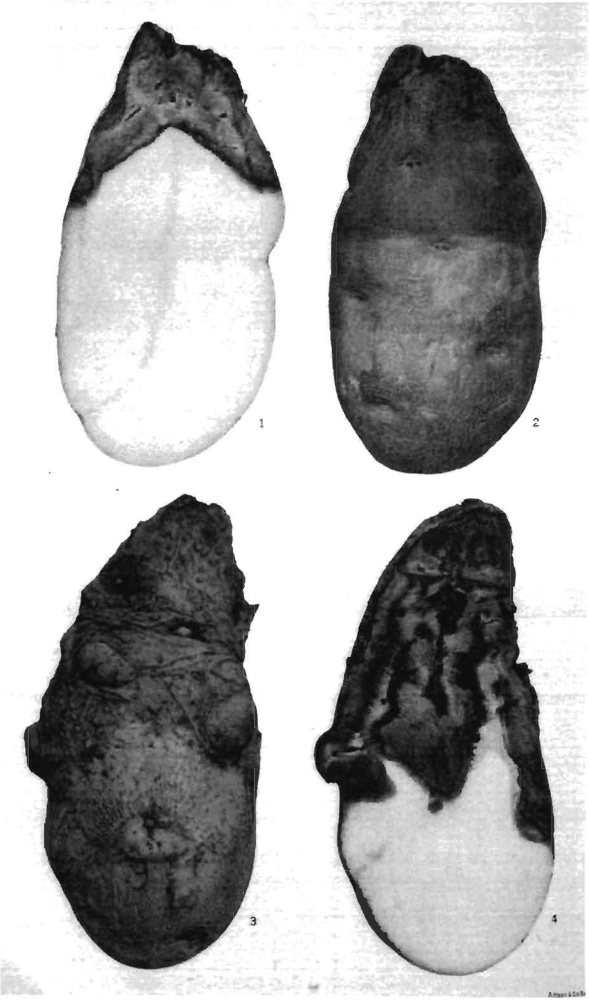


PLATE A

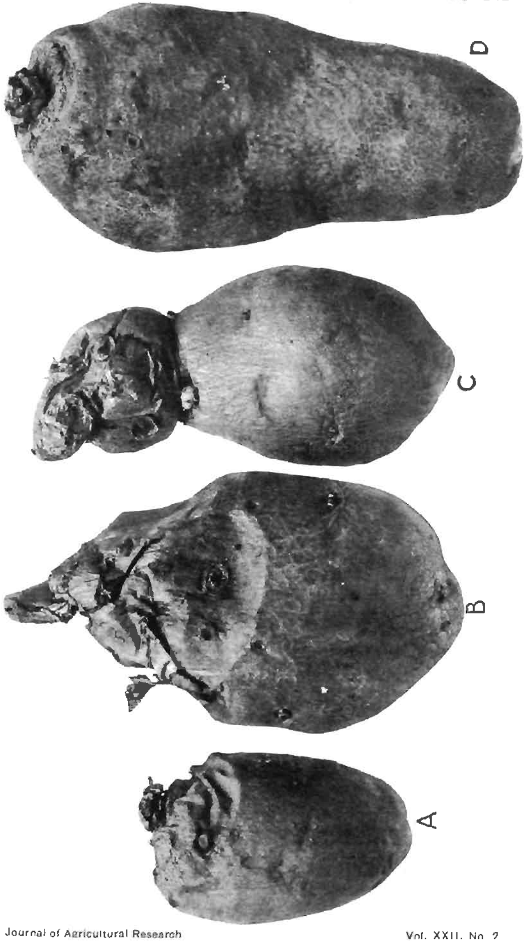
Types of blackleg potato tuber-rot on pointed-end Netted Gem from Idaho, showing external as well as internal appearance. The photograph was taken two weeks after the tubers were removed from the ground. The same tubers are shown in Plate 15, A, C, on the first day after digging.

PLATE 12

Forms of blackleg tuber-rot in the West.

A–C. — Specimens received in August, 1918, from Fresno, Calif.

D. — Specimen received in December, 1917, from Fallon, Nev.


D
C
B
A

B
A

PLATE 13

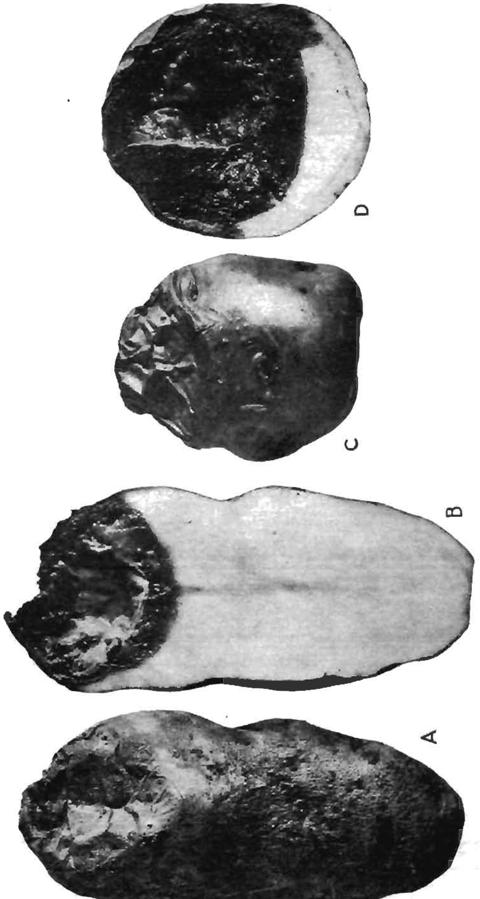
Blackleg on stems resulting from planting the diseased western material.
A.—Appearance of plant above the grownd.
B.—Spread of the infecion from the diseased seed piece to the new stem.

PLATE 14

Result of inoculation of healthy tubers with the bacterial organism isolated from the western diseased material.

A, B.—Netted Gem variety.

C, D.—Irish Cobbler variety.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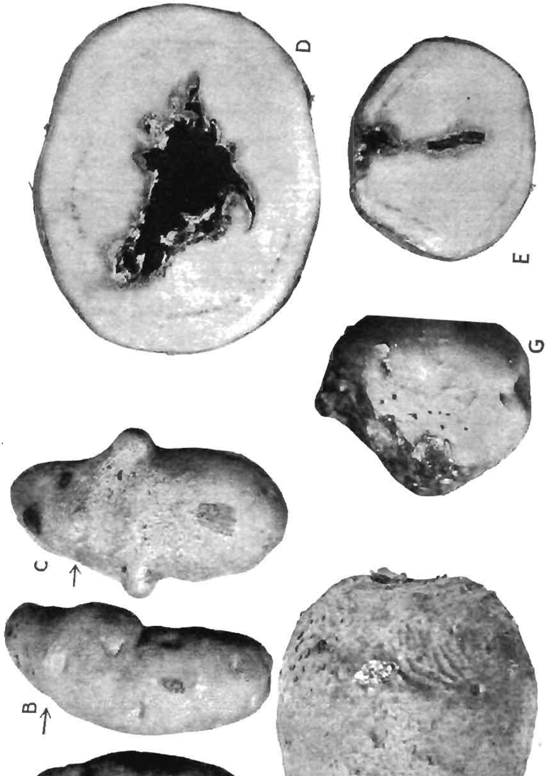

A
B
C
D
E
F
G

PLATE 15

Different types of blackleg tuber-rot.

A–C.—Characteristic appearance on fresh specimens of the Netted Gem variety from Idaho.

D, E.—Typical development on round varieties in the East.

F, G.—Other forms occurring on round varieties in various sections of the country.

Arrows in A, B, and C indicate the border line of decay.

PLATE 16

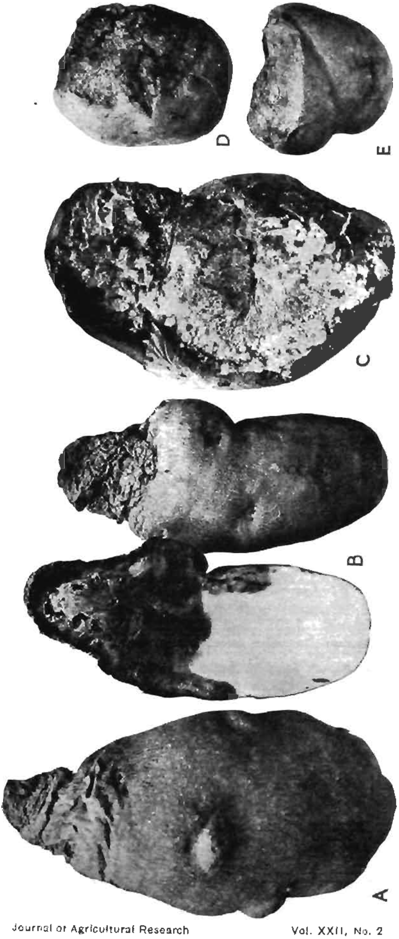
Confusing forms of blackleg potato tuber-rot in the West (all specimens collected in Idaho).

A, B.—Netted Gem variety one month after digging. Tuber B as it appeared on the first day after digging is shown in Plate 15, B.

C.—Long Idaho Rural, showing secondary growth of Rhizoctonia on the outside and active bacterial decay in the inside.

D.—Idaho Rural with deep side infection of blackleg decay which became dry and spongy on exposure to the sun.

E.—Shallow surface infection which became dry and black when exposed to the sun after digging.


A
B
C
D
E

# MICROSCOPIC STUDY OF BACTERIA IN CHEESE

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## INTRODUCTION

Heretofore cultural methods have ordinarily been used in the study of cheese flora, but the usual routine technic has given only an inadequate conception of the number and types of bacteria present. Although these cultural methods were employed in order to secure an idea of the number and varieties of organisms occurring in cheese, they have failed to establish the relative abundance of each type of microorganism in the cheese. This objection remains valid in the light of both quantitative and qualitative studies.

The so-called "dilution technic," which involves the mass action of the organisms, has proved valuable in determining the type or group of organisms which predominates in a given sample; but it does not furnish information regarding the general flora. This method often tends to give erroneous results, especially when the dilution medium used favors the growth of special groups. Under such conditions the results are influenced by the selective action of the medium. This has been true where milk was used as a dilution medium in examining cheese. The milk favored the growth of the lactic acid group; while the inert and nonlactose fermenting types or slow-growing cocci were overgrown, due in a large measure to the selective action of the medium. This procedure has well served its purpose in assisting to isolate the organisms for which it has a special adaptation.

In general, cultural methods are preferable to a microscopic examination because cultures can be isolated and studied independently—a feature which will always remain the outstanding advantage of these methods.

Although subject to the same limitations as any microscopic method, the following method has been successfully used in this laboratory and has been employed in a routine way in determining the number of bacteria in cheese.

## HISTORY

Johan-Olsen (*8*)[1], working with the molds which ripen "Gammelost" (a Norwegian cheese), mentions a sectioning method and implies that it resembles the usual histological technic but does not outline the procedure in detail.

[1] Reference is made by number (italic) to "Literature cited," p. 100.

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Troili-Peterson (*11*), in discussing the bacterial flora of Swedish "Güterkäse," mentions the microscopic examination of cheese as a control for the cultural procedure but does not give the technical details. She presents photomicrographs of cheese sections and states that some of the preparations were stained in methylene blue and that a few were examined unstained.

Gorini (*3*), in studying the distribution of the bacteria in Grana cheese, presents the details of a method by which he prepared sections for microscopic examination. In his procedure he fixed and dehydrated samples of cheese by passing them through a series of alcohols of increasing concentrations until a strength of 95 per cent was reached. The usual histological methods of sectioning were followed, and the sections were stained in an aqueous solution of methylene blue.

In the following year Rodella (*9*) reported a method used in his laboratory for preparing sections used in the direct examination of cheese samples. With his technic the samples were dehydrated and fixed by a method similar to that of Gorini and sectioned in the usual way. He found, however, that carbol-thionin gave better results as a stain than did methylene blue.

Harrison (*6*) outlined in detail a method for embedding and sectioning cheese which is similar to the common histological method, but like his predecessors he made no estimate of the number of bacteria present.

During the year in which Rodella (*9*) presented his paper, Troili-Peterson (*12*) and Gorini (*4*) published notes discussing the question of priority raised by the practically simultaneous publication of their papers. It appears that the methods followed by Trioli-Peterson were similar to those of Gorini, but that she did not feel the necessity of presenting the technical details because of the universal knowledge of the common embedding methods.

No results have been obtained in any of this work that permit a comparison between counts made by the plate method, so commonly used in floral studies of cheese, and counts made by direct microscopic examination. Following the method outlined below, comparatively accurate counts have been made by the direct method, and the number of the different types of bacteria have been determined as they actually exist in the cheese mass.

## TECHNIC

### EMBEDDING AND SECTIONING

The samples of cheese were embedded by the usual histological technic and sectioned with a Minot rotary microtome. In sectioning, the microtome was so adjusted as to give sections 5 $\mu$ thick. The sections were stained by the Gram method and with an aqueous solution of methylene blue.

In order to determine the effect of the embedding process upon the cheese, small measured cubes of cheese were subjected to the routine procedures. Only a slight shrinkage was found, indicating that the volume of embedded cheese when examined is approximately the same as that of the fresh sample.

## MICROSCOPIC EXAMINATION

The preparations were examined with an oil immersion lens and a high power ocular, the most satisfactory combination being a 1.9-mm. fluorite objective with a numerical aperture of 1.32. Where a thick coverslip was used it was necessary to have a 3-mm. apochromatic objective with a numerical aperture of 1.4. Greater depth can be secured with compensating oculars than with the ordinary Huygenian oculars.

The method, although at first used only for determining the types of organisms present in the samples and as a check on the usual plate method, was found useful as a means of determining the number of organisms present. In order to make such a computation the microscope was so standardized as to allow an estimate of the number of organisms per gram when only a small amount of the original section was examined. This computation is similar to that used in the direct method of counting bacteria in milk described by Breed and Brew (2). This was accomplished by measuring both the diameter of the microscopic field and the thickness of the section from which the amount of cheese actually seen in each field examined was determined. Knowing the volume and specific gravity of the cheese examined, the total number of organisms per gram can readily be computed. With the diameter of the field measuring 0.14 mm. (140 $\mu$), the microtome so adjusted as to cut sections of a thickness of 0.005 mm. (5 $\mu$), and a specific gravity of 1, the amount of cheese examined per microscopic field would be 1/13,000,000 gm.—that is, each organism observed in a single microscopic field represents 13,000,000 per gram.

This factor may be computed by the following formula, in which any measure may be substituted:

$$\frac{1{,}000}{\pi r^2 a} b = \text{factor per gram.}$$

In the above formula,

$r$ = the radius of the field examined in millimeters as determined by actual measurement.

$a$ = the thickness of the section in millimeters.

$b$ = the specific gravity of the cheese.

The radius of the field, as has been stated, is determined by measurement with a stage micrometer and varies with the magnification and with the type of ocular used. However, it was found advisable to adjust the

draw tube of the microscope so that the field would be of the greatest possible diameter without losing definition, as the greater the diameter of the field the less the increment of error in the total counts.

The thickness of the section is controlled by adjusting the microtome to cut sections of a desired and known thickness. If all the adjustments on the microtome are firm and a sharp knife is used, sections can be cut of uniform thickness with surprising accuracy. The thickness of the sections can also be remeasured with the fine adjustment screw on the microscope. Although not perfect, this method of measurement serves as a check upon the accuracy of the sectioning. The measurement is accomplished by focusing with the graduated fine adjustment screw on both the upper and lower surfaces of the section and noting the differences in the readings between the two levels. The difference can be read in microns where graduations are given on the fine adjustment screw.

To convert the per-cubic-centimeter counts into numbers per gram, the specific gravity of the cheese must be considered. As the specific gravity of all samples has been assumed to be approximately 1, the counts are interchangeable. This assumption in regard to the specific gravity is arbitrary, but the variations in the specific gravity of cheddar cheese are so slight that the total count is not affected to any appreciable degree. Accurate determinations did not seem practicable, as the specific gravity varies with the fat content and with the moisture and general consistency of the cheese.

With the measurements and adjustments used in this laboratory the per-gram formula resolves itself into the following:

$r = 0.07$ mm. ($70\ \mu$).

$a = .005$ mm. ($5\ \mu$).

$b = 1.0$.

$$\frac{1{,}000}{3.1416 \times 0.0049 \times 0.005} \times 1 = \text{approximately } 13{,}000{,}000.$$

## APPLICATION OF THE METHOD

It is evident that this microscopic technic is subject to the limitations of any direct method of examination, many of which are unavoidable and are due to mechanical limitations or to the human error, which enters in when counts or estimates are made.

### QUALITATIVE EXAMINATION

As previously stated, cheese has been examined microscopically by many investigators. The possibility of error is not as great when samples are examined to determine the types of organisms present as when total count is made, which is true of any microscopic work. Our present staining methods make possible a direct visualization of the microorgan-

isms together with their morphological and other general characteristics, but an attempt to enumerate these types involves other difficulties.

The direct examination of cheese in the different ripening stages is advantageous and important, since the different groups of organisms can be studied as they actually occur in the cheese mass, and their groupings and relative relationships noted. The grouping may be especially important when considered in relation to the number present. For example, an organism may be present in large numbers during the early stages of ripening, but appear in scattered and isolated groups containing only a few individuals. In some instances only single bacteria were found through the mass. In such cases the total number of this group by the plate count may be large, but the grouping, as determined by direct examination, may demonstrate that they are not actively growing and playing a part in the ripening of the cheese. On the other hand, the presence of large clumps of organisms, with the size of the clumps increasing during ripening, indicates that such groups are developing in the cheese mass and are probably playing an important rôle in the changes involved.

That this grouping of the organisms actually occurs can be seen in Plate 17, A. In this photomicrograph are shown the types and groupings of organisms found in a very green cheese, showing that the *Streptococcus lactis*-like organisms predominated and were scattered in pairs over the field. Any migration of these bacteria through the cheese mass appears to have been impossible, and one is impressed with the fact that growth and reproduction could not have been taking place rapidly or the number of individuals per group would have been larger. In Plate 17, B, which represents a section from a cheese 5 months older than that shown in Plate 17, A, the organisms are found in larger clumps with many of them so massed that accurate counting is impossible. From the examination of a series of sections from cheeses of varying ages, it has been found that the clumps increase in size as the cheese ripens, reaching a limit after seven to eight months. It is evident that the organisms in the clumps, mostly cocci and a few rods, are thriving and reproducing and must, therefore, change the surrounding medium as they utilize it for food. It is not within the scope of this paper to discuss the significance of this occurrence but only to point out that such variations are found when samples are examined directly.

## QUANTITATIVE EXAMINATION

An objection often made to counting organisms in microscopic preparations of dried liquids is the uneven thickness of the resultant dried film. This objection is eliminated when paraffin sections are used, as such sections are uniform in thickness and the organisms remain in their natural relationships. Boekhout and DeVries (*1*) at one time endeavored

to show that the scattered organisms in cheese sections were due, in a large measure, to the breaking up and scattering of the clumps by the knife edge. This explanation will hardly appear plausible to anyone familiar with the perfection of delicate histological sections prepared with a sharp knife.

The grouping and clumping of the organisms often cause difficulty in accurately determining the number of organisms in the cheese sections. This is especially true in sections of old cheeses in which the bacteria tend to clump in large masses. The error can be overcome to a large degree by counting or estimating a large number of fields, the larger the number examined the smaller being the error in the final estimate.

In a sample of green cheese where the organisms appear in large numbers, but are evenly scattered, it is impracticable to count the entire field, and an ocular disk divided into quadrants may be inserted in order to facilitate accurate counting.

In all cases 20 or more fields should be counted, and especially where the organisms are unevenly distributed. In such instances, typical fields which represent the general flora should be located by studying the entire section.

## COMPARISON OF DIRECT AND PLATE COUNTS

Table I gives a few representative comparisons between direct microscopic and plate counts made from cheese samples in various stages of ripening. The plate counts average approximately one-twelfth the direct count, but no common ratio has been found to exist between the results obtained by the two methods. Wide variations in the ratios between the counts were found, but in general the ratios from green cheese appeared to be larger than those from cheese more advanced in ripening.

The above plate counts compare well with those found by other observers who have examined cheddar cheese, Russell (*10*) found from 62 to 665 million per gram. Harrison and McConnell (7) found the count to be as high as 625 million per gram in the earlier stages of the ripening, while Harding and Prucha (5) observed from 37 to 177 million per gram.

Several explanations may be offered to account for the apparent discrepancy between the results obtained by the two methods. The plate count is an estimate based on observations of the growth of organisms on some particular medium which, in cheese investigations, usually contains lactose. Lactose has been generally used because media containing this particular carbohydrate have been found to allow the development of a larger number of colonies than do sugar-free media. Investigators have based their cultural methods upon media giving the largest counts rather than upon media which might serve as an index to the relative number of types present. In comparing the microscopic counts with results obtained with the plate method, it may be noted that the types

present in the cheese, as seen by direct examination, are not present in the same proportions on the plates, because those types which grow abundantly in the presence of lactose have outnumbered all groups which do not grow as readily on such media.

TABLE I.—*Relation between microscopic and plate counts obtained from cheese*

| Cheese. | Approximate age of cheese. | Miscroscopic count (millions per gram). | | | | | Plate count (millions per gram). | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Cocci. | Short rods. | Yeast | *Streptococcus lactis.* (Lister) | Total. | *Streptococcus lactis.* | Miscellaneous. | Total |
| OOCH XII 2 | 2 months. | 100 | 600 | . | 4,500 | 5,200 | 46 | 9 | 55 |
| OOCI3 XII 2 | 2 months. | 14,650 | 5,200 | .. | 21,450 | 41,300 | 264 | 284 | 548 |
| 6, 4, II | 6 months | 312 | | 26 | 143 | 481 | 132 | 90 | 222 |
| B C II | 3 months. | 1,690 | 780 | . | 6,760 | 9,230 | 251 | 41 | 292 |
| 6, 26, II | 5 months, 23 days | 962 | 260 | | 988 | 2,210 | 306 | 121 | 427 |
| OO3SIS | Unknown; appeared green | 338 | 20 | . | 3,029 | 3,393 | . | | 702 |

Results of plate counting may also be lower because of insufficient grinding and emulsifying of the cheese sample previous to plating. This appears to be especially significant in cases where investigators grind the sample with sterile quartz or sugar and suspend the ground mass in sterile water in preparation for plating. If the sample is not well ground, small particles of cheese remain in the emulsion, and the individual bacteria are not separated so as to allow them to grow into separate colonies on the artificial medium. Emulsions examined under the microscope often show comparatively large masses of cheese which have not been affected by the grinding process.

## CONCLUSION

The microscopic examination of cheese embedded and sectioned by the usual histological method is a valuable and satisfactory method for studying the different stages of cheese ripening. Such a direct method of examination may be used to determine the number of organisms present in the sample. It also serves as an index to the types of organisms present and makes possible a study of the organisms as they actually exist in the cheese mass, allowing observations on the groupings and relationships during cheese ripening.

The cultural methods do not yield as high a count as the microscopic method, due primarily to the selective action of the medium used and the difficulty of liberating the organisms from the cheese mass previous to plating.

A combination of microscopic and cultural studies yields a far more complete picture of what takes place in cheese ripening than can be obtained by the use of either method alone.

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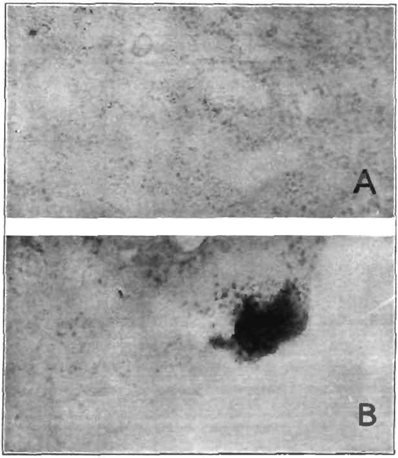
PLATE 17.

A.—Section of cheddar cheese 1 month old, stained with an aqueous solution of methylene blue, showing isolated pairs of *Streptococcus lactis* Lister throughout the field. × 500.

B.—Section of cheddar cheese 6 months old, stained as in A. × 800.



# FURTHER STUDIES ON RELATION OF SULPHATES TO PLANT GROWTH AND COMPOSITION

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This is a continuation of an investigation, part of the results of which were reported in a former publication (*4*).[1] As stated in the earlier paper, the addition of the different forms of sulphur caused a marked increase in the dry weight of red clover, and beneficial results were obtained with oats and rape. One very noticeable result observed in the former work was the high nitrogen content of the clover grown on soils in the greenhouse receiving sulphur fertilizer, compared to that of clover receiving only the residual sulphur of the soil.

This great increase in nitrogen assimilation by the clover where sulphates were applied, and under the conditions described, led the writer to believe that the sulphates favorably influenced the activity of the legume bacteria. Especially did this appear true where the beaverdam soil was used. This soil contained 0.18 per cent sulphur with appreciable quantities of sulphate sulphur in the soil extract, and no beneficial result from sulphur fertilizer was expected. Oats did not respond to sulphur with this soil, although the sulphur content of oats (*3*) and the amount of sulphur removed by one crop of oats is as large as with a red clover crop. From present data, the responses of red clover so often obtained with gypsum compared to cereals can not be explained through a difference in sulphur requirement. With alfalfa the amount of sulphur removed is so large compared to the cereals and red clover that the addition of sulphates would apparently function directly as a plant food where increased growth results. An example of the latter would be the enormous increases in the yield of alfalfa obtained in southern Oregon (7) where sulphur fertilizers were applied to soils with a very low sulphur content. These authors, however, mention the favorable action that sulphur fertilizers had on the root development and nodule production of alfalfa. Duley (*2*) reports increase nodule production on red clover where sulphur was added to soils. Pitz (*5*) observed increased nodule production and root development with red clover by applying gypsum to soil cultures.

As far as the writer can ascertain, no correlation has been shown between nodule production and nitrogen content of the plant, by influencing the development of the former, with ordinary sulphur fertilizer compounds. In this paper a study has been made of the effect of different

[1] Reference is made by number (italic) to "Literature cited," p. 110.

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concentration of sulphates on growth and nitrogen assimilation, and also the relation of total sulphur content of the plant as influenced by available nitrogen. The red clover and rape were used in this work. With clover, the initial concentration of legume bacteria has been varied by inoculating certain cultures, while others were not inoculated.

TABLE I.—*Analytical results with red clover on Medford loam soil*

| Treatment | Weight of air-dried clover. | Total N. | N insoluble in acetic acid. | Total S. | Sulphate S. | Organic S. | S in acetic-acid solution. | S insoluble in acetic-acid solution | Weight of air-dried roots. | Total S in roots. | Total N in roots. | Ratio of tops to roots. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Gm.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* | *Gm.* | *P. ct.* | *P. ct.* | |
| Control: | | | | | | | | | | | | |
| Uninoculated.. | 15.84 | 2.85 | 2.13 | 0.20 | 0.00 | 0.14 | 0.10 | 0.10 | 6.00 | 0.32 | 1.69 | 2.64 |
| Inoculated | 21.24 | 3.31 | 2.10 | .20 | .04 | .16 | .08 | .12 | 7.65 | .34 | 2.16 | 2.77 |
| $NaNO_3$: | | | | | | | | | | | | |
| Uninoculated. | 17.70 | 2.81 | 2.02 | .18 | .07 | .11 | .08 | .10 | 6.45 | .28 | 1.64 | 2.74 |
| Inoculated | 21.29 | 3.11 | 2.10 | .16 | .02 | .14 | .07 | .09 | 7.80 | .23 | 1.83 | 2.73 |
| $NaNO_3$ and $NaSO_4$ | | | | | | | | | | | | |
| Uninoculated | 18.82 | 3.30 | 2.15 | .26 | .10 | .10 | .15 | .11 | 7.60 | .60 | 1.91 | 2.48 |
| Inoculated | 23.35 | 3.16 | 2.22 | .28 | .12 | .16 | .17 | .11 | 7.10 | .72 | 1.91 | 3.29 |
| $Na_2SO_4$: | | | | | | | | | | | | |
| Uninoculated. | 15.00 | 3.58 | 2.31 | .40 | .25 | .15 | .31 | .09 | 8.60 | .59 | 1.73 | 1.74 |
| Inoculated.. | 20.31 | 3.48 | 2.19 | .34 | .14 | .20 | .20 | .14 | 8.20 | .53 | 2.17 | 2.47 |
| $CaSO_4$: | | | | | | | | | | | | |
| Uninoculated. | 16.49 | 3.46 | 2.39 | .28 | .13 | .15 | .35 | .13 | 7.20 | .50 | 2.04 | 2.29 |
| Inoculated | 16.05 | 3.38 | 2.40 | .34 | .15 | .19 | .20 | .14 | 5.70 | .61 | 2.05 | 2.92 |
| $NaNO_3$ and $CaSO_4$. | | | | | | | | | | | | |
| Uninoculated. | 14.18 | 3.33 | 2.28 | .41 | .27 | .14 | .28 | .13 | 6.10 | .55 | 1.75 | 2.32 |
| Inoculated.. | 16.89 | 3.34 | 2.33 | .33 | .15 | .18 | .18 | .15 | 6.20 | .55 | 2.00 | 2.72 |

In the first experiment Medford loam soil, designated as soil B in the previous publication (*4*), was used. This soil was heated in an electric oven where the temperature was gradually raised to 120° C. and maintained for six hours. This was to destroy the legume bacteria present in the soil. Four kgm. of soil, after being mixed with the different fertilizers, were placed in paraffined clay pots and carefully seeded to red clover. One series was inoculated with *Bacillus radicicola* Bey. The solution for inoculation was prepared by removing the growth of organisms from a culture and mixing with water. Each inoculated soil culture received a definite number of cubic centimeters of the bacterial solution, concentrated where the seeds were placed. The amount of different fertilizers added per pot was as follows: Sodium sulphate ($Na_2SO_4$), 3 gm.; sodium nitrate ($NaNO_3$), 2 gm.; calcium sulphate ($CaSO_4.2\ H_2O$), 3.75 gm.; and calcium carbonate ($CaCO_3$), 3 gm. The cultures were placed in the greenhouse on October 16 and harvested on March 18. Ten plants were allowed to grow in each pot. The cool temperature in the greenhouse did not permit rapid growth, and the plants were cut before maturity was reached. The treatment and analytical results are given in Table I. The cultures were weighed every other day, and the moisture was maintained at 20 per cent. In removing the roots the soil was shaken out of the pot and carefully loosened. The roots were then separated out and washed. After drying they were weighed, and the non-

volatile matter was determined by ashing a ground sample representing each culture. This was done to correct for any excess weight due to adhering soil particles.

The total sulphur was determined by the sodium-peroxid method. The sulphate sulphur was extracted by taking 2 gm. clover and 150 cc. of water and digesting on the steam bath for three hours. It was then slightly acidified with hydrochloric acid, and after standing for an hour the extract was filtered. Five cc. of 10 per cent barium-chlorid solution was used to precipitate the sulphate sulphur in the hot solution. After standing overnight the barium sulphate settled to the bottom of the beaker in all cases, and no particles of precipitate could be detected in the supernatant liquid. This liquid was carefully decanted off; and the white precipitate was washed on a Gooch crucible, dried, and weighed. Several of these precipitates were ignited, but since no appreciable loss in weight was detected this method appeared perfectly reliable for comparable results on sulphate sulphur in the different samples of plant material. For the determination of total nitrogen insoluble in acetic acid, 1-gm. samples were digested with about 150 cc. of water on the water bath for two hours. The extract was then acidified with dilute acetic acid and filtered after standing about 30 minutes. The total nitrogen was determined on the precipitate by the Kjeldahl method. The filtrate was made alkaline with sodium carbonate, evaporated to a few cubic centimeters in volume, transferred to a nickel crucible, and total sulphur determined by the sodium-peroxid method.

The dry weights of plant material produced show no increase in production that can be attributed to presence of sulphates. This result is different compared to the noticeable increase reported with the same soil before (4). As mentioned above, conditions were very unsatisfactory for growth, and the plants were cut before maturity five months after planting. In the former work reported, conditions permitted rapid growth, and the plants, though not mature, were harvested two months after seeding. As the soil used in this later work had been heated, there was perhaps some change in degree of solubility of soil minerals and in the biological flora. The concentration of added mineral salts was also greater in this work.

Examination of the roots showed that all plants had become infected. In the uninoculated series, roots from cultures 1 and 2 contained very few nodules compared to the roots grown in the soil receiving sulphate fertilizer. This remarkable difference in nodule formation no doubt accounts for the low nitrogen content of the clover plants in pots 1 and 2. That these plants became infected without any artificial inoculation is not surprising. Wilson (8) found that—

of fifteen legumes grown in Volusia silt loam soil, only one, Trifolium pratense, developed nodules without artificial inoculation.

During the growth of the plants the inoculated series showed greater development, which is apparent upon examining the dry weights. Cultures 1 and 2 of the inoculated series show no effects from lack of sulphates, and all the sulphur-fertilized pots contained numerous well-developed nodules.

The total nitrogen insoluble in acetic acid was no greater in some of the cultures receiving added sulphates than in the controls, so no statement can be made that sulphate addition caused this fraction to become larger. The nitrogen content of the fraction soluble in acetic acid is lower in 1 and 2 of the uninoculated series. The increase in percentage of sulphur caused by fertilizer treatment is accounted for generally by higher sulphate content. Although the organic sulphur is apparently higher in some, the results are not consistent with the total sulphur, to state that the former results from increased sulphur assimilation in this experiment. Total sulphur in the acetic-acid extracts runs parallel with sulphate sulphur results and is slightly higher, showing that there is some sulphur in the organic form not accounted for in the precipitate from acetic-acid solution. This was also found to be true with clover grown in other pots which was harvested while in blossom.

The ratio in weight of tops to roots is greater in the inoculated sulphur-fertilized cultures than in the uninoculated sulphur-fertilized cultures. This difference does not appear to be due to inoculation alone, for this does not hold true in comparing 1 and 2 of both series, while the ratio of tops to roots in 1 and 2 of the uninoculated series is greater than the remaining four where there is heavy nodule growth. Arny and Thatcher (*1*) report a greater ratio in weight of tops to roots where inoculation was made with alfalfa and sweet clover.

The sulphur content of the roots is larger than in the other portion of the plant, whereas the opposite is true in percentage of nitrogen.

The second part of this work was carried on with beaverdam soil and red clover. Each pot contained 7 kgm. of soil, and the following amount of fertilizers were added as indicated in Table II: 12 gm. of calcium sulphate, 10 gm. of sodium sulphate, 2 gm. of sulphur, and 6 gm. of sodium nitrate. Two gm. of potassium chlorid and 10 gm. of calcium carbonate were added to all the soil cultures. Twenty red clover plants were allowed to grow in each pot, and the moisture content was kept at 60 per cent. The first crop grew at the same time as the clover on the Medford loam soil and was also cut before the blossoming stage. Three other crops were grown on these same cultures. The first was harvested on March 24, the second on May 20, the third on July 9, and the fourth on August 17. The last three crops were cut during the blossoming stage. The results are given in Table II.

TABLE II.—*Analytical results with red clover on beaverdam soil*

| Treatment. | Crop I. | | | Crop II. | | | Crop III. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Weight of air-dried clover. | Total N. | Total S. | Weight of air-dried clover. | Total N | Total S. | Weight of air-dried. clover. | Total N. | Total S | Sulphate S | Organic S |
| | *Gm.* | *P. ct.* | *P. ct.* | *Gm* | *P. ct* | *P. ct* | *Gm* | *P. ct* | *P. ct* | *P. ct* | *P. ct.* |
| Control | 46 | 3.28 | 0.21 | 94 | 2.55 | 0.14 | 69 | 2.43 | 0.14 | 0.02 | 0.12 |
| $CaSO_4$ and $NaNO_3$ | 43 | 3.50 | .21 | 92 | 2.70 | .20 | 66 | 2.67 | .22 | .. | . |
| $CaSO_4$ | 46 | 3.41 | .21 | 84 | 2.50 | .20 | 52 | 2.67 | .21 | .06 | .15 |
| $NaNO_3$ | 53 | 3.25 | .19 | 102 | 2.40 | .14 | 57 | 2.39 | .14 | .02 | .12 |
| $Na_2SO_4$ | 44 | 3.47 | .21 | 91 | 2.53 | .16 | 52 | 2.72 | .20 | .07 | .13 |
| $Na_2SO_4$ and $NaNO_3$ | 45 | 3.12 | .22 | 80 | 2.49 | .20 | 58 | 2.78 | .20 | ... | . |
| S | 45 | 3.42 | .22 | 85 | 2.47 | .17 | 58 | 2.65 | .21 | ... | . |
| S and $NaNO_3$ | 46 | 3.58 | .23 | 88 | 2.56 | .16 | 63 | 2.72 | .21 | ... | . |

| Treatment. | Crop IV | | | | |
|---|---|---|---|---|---|
| | Weight of air-dried clover. | Total N | Total S. | Sulphate S. | Organic S. |
| | *Gm.* | *Per cent* | *Per cent* | *Per cent.* | *Per cent.* |
| Control | 27 | 2.88 | 0.18 | 0.03 | 0.15 |
| $CaSO_4$ and $NaNO_3$ | 28 | 3.10 | .30 | .15 | .15 |
| $CaSO_4$ | 24 | 3.15 | .27 | .12 | .15 |
| $NaNO_3$ | 24 | 2.89 | .22 | .06 | .16 |
| $Na_2SO_4$ | 27 | 3.01 | .27 | .11 | .16 |
| $Na_2SO_4$ and $NaNO_3$ | 29 | 3.22 | .29 | .13 | .16 |
| S | 29 | 3.16 | .28 | .14 | .14 |
| S and $NaNO_3$ | 26 | 3.10 | .28 | .12 | .16 |

Examination of the foregoing data shows no result from sulphate application in the first two crops. There is no increase in dry weight in the pots receiving sulphur fertilizers, and in some crops the yield on the control is greater. In the third and fourth crops, one distinctive difference appears in the lower nitrogen content of the clover grown on the control soil cultures. The fact that the clover grown under conditions supplying more sulphate sulphur has a higher nitrogen content adds to the data already obtained pertaining to the favorable influence that sulphates have upon legume bacteria, the action of which results in a higher nitrogen content of the clover. According to experimental evidence, sulphates do not increase nodule production on all legumes. Wilson (*8*) reports that certain sulphates depressed nodule formation on the soybean. On the other hand, Prucha (*6*) mentions magnesium sulphate and calcium sulphate as exerting a beneficial influence on nodule development of the Canada field pea.

The low nitrogen content did not appear to be due to the absence of sulphates in the plant tissue, thus limiting protein synthesis, as sulphates were present in all samples. However, the percentage of sulphate sulphur was lower in clover grown on the control pots.

That the presence of available nitrogen or nitrogen assimilation by the plant tends to control or limit the total sulphur assimilation is illustrated by data in Table III.

In this experiment sea sand was used which had been washed with dilute hydrochloric acid and large volumes of distilled water. The sand still contained sulphur compounds, but no sulphates soluble in dilute hydrochloric acid. Six kgm. of sand were mixed with 10 gm. of calcium carbonate and placed in paraffined clay pots. Where elemental sulphur was used, 0.75 gm. was added at the same time. The other nutrients added were applied in solution form through a period of 70 days; the growing period was 80 days. The total amount of sodium sulphate which was added varied in the different cultures. Concentration 1 denotes 1.55 gm., concentration 2, 3.10 gm., etc. The same figures apply to calcium sulphate too. All cultures, with the exception of No. 3, 13, 20, and 21, received 3.9 gm. of sodium nitrate, and they each received 0.75 gm. Every culture received 2.6 gm. of potassium dihydrogen phosphate and 1.3 gm. of magnesium chlorid. Twenty plants grew in each pot, and the moisture content varied fron 15 to 20 per cent in the different cultures. The weights recorded are the average of duplicates, and the analyses were made on a sample obtained by mixing the duplicates. The 22 cultures from 11 to 21, inclusive, were inoculated with legume bacteria and 5 gm. of beaverdam soil added to the same culture.

TABLE III.—*Analytical results of clover grown on sand receiving a nutrient solution*

| Uninoculated series | | | | | | Inoculated series | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Culture No. | Form of sulphur added | Sulphate concentration. | Weight of air-dry clover. | Total S. | Total N. | Culture No. | Weight of air-dry clover. | Total S. | Total N. | Sulphate S. | Organic S. |
| | | | *Gm.* | *Per ct.* | *Per ct.* | | *Gm.* | *Per ct.* | *Per ct.* | *Per ct* | *Per ct.* |
| 1 | $Na_2SO_4$ | 1 | 9.65 | 0.26 | 3.76 | 11 | 10.00 | 0.27 | 3.62 | 0.12 | 0.15 |
| 2 | ....do | 3 | 10.7 | .42 | 3.77 | 12 | 7.00 | .30 | 3.71 | .12 | .18 |
| [a] 3 | ....do | 1 | 3.95 | .30 | 2.00 | [a] 13 | 4.80 | .20 | 2.49 | .08 | .12 |
| 4 | $Na_2SO_4$ and $CaSO_4$ | 2 | 8.75 | .30 | 3.50 | 14 | 6.45 | .32 | 3.70 | .18 | .14 |
| 5 | $Na_2SO_4$ | 2 | 6.9 | .34 | 3.69 | 15 | 6.85 | .29 | 3.60 | .15 | .14 |
| 6 | Control | ...... | 4.15 | .35 | 3.90 | 16 | 7.3 | .27 | 3.90 | .08 | .19 |
| 7 | S | | 6.62 | .27 | 3.75 | 17 | 7.77 | .32 | 3.69 | .17 | .15 |
| 8 | $CaSO_4$ | 2 | 7.02 | .34 | 3.85 | 18 | 7.30 | .32 | 3.70 | .18 | .14 |
| 9 | do | 1 | 5.3 | .34 | 3.80 | 19 | 5.90 | .30 | 3.90 | ...... | ...... |
| 10 | ...do | 1 | ...... | ...... | ...... | [a] 20 | 7.50 | .20 | 2.42 | .09 | .11 |
| 11 | $Na_2SO_4$ and $CaSO_4$ | 2 | ...... | ...... | ...... | [a] 21 | 5.07 | .26 | 2.46 | .14 | .12 |

[a] Low nitrate.

In the foregoing data the low sulphur contents occur in the clover grown in the pots receiving less nitrate nitrogen. In the inoculated series the sulphur content of the clover does not appear to increase by increasing the sulphate sulphur of the nutrient media. On the other hand, where there is a reduction in nitrate added, there is an appreciable reduction in the sulphur content of the clover. In the other series, where the concentration of legume organisms was not as great at the start, the percentages of sulphur generally run higher. In No. 3 the percentage of

sulphur is not lower than in some of the others, but here the total yield is small, and this often accounts for higher percentages of certain elements. The yields in No. 3 and 6 are about the same; but the sulphur content is higher in 6, although this culture depended only upon the sulphur in the sand. No. 2 shows response in sulphur content to the higher concentration of sulphates in the media. The corresponding culture 12 in the other series does not show higher sulphur content; and as the average sulphur content is lower in this uninoculated series, it appears that the legume organisms might have some effect on limiting the quantity of sulphur present in the clover hay.

TABLE IV.—*Data showing the sulphur-nitrogen relation in the portion insoluble in dilute acetic acid*

| | In soil treated with— | | | | | Ratio of N to S in the insoluble portion. | Average ash content. |
|---|---|---|---|---|---|---|---|
| | $CaSO_4$ and $NaNO_3$. | $NaNO_3$. | $Na_2SO_4$. | $Na_2SO_4$ and $NaNO_3$. | Average. | | |
| Crop I: | | | | | | | |
| Percentage of N insoluble in acetic acid | *Per ct.* 2.26 | *Per ct.* 2.24 | *Per ct.* 2.58 | *Per ct.* 2.15 | *Per ct.* 2.31 | 17.7 | *Per ct.* 9.94 |
| Percentage of S soluble in acetic acid | .08 | .07 | .09 | .07 | ...... | | |
| Percentage of S insoluble in acetic acid | .13 | .12 | .12 | .15 | .13 | | |
| Crop II: | | | | | | | |
| Percentage of N insoluble in acetic acid | 1.70 | 1.57 | 1.64 | 1.73 | 1.66 | 18.8 | 7.31 |
| Percentage of S soluble in acetic acid | .11 | .06 | .07 | .11 | | | |
| Percentage of S insoluble in acetic acid | .09 | .08 | .09 | .09 | .088 | | |
| Crop III: | | | | | | | |
| Percentage of N insoluble in acetic acid | 1.78 | 1.76 | 1.87 | 1.85 | 1.81 | 19.4 | 6.95 |
| Percentage of S soluble in acetic acid | .13 | .05 | .10 | .11 | ...... | | |
| Percentage of S insoluble in acetic acid | .09 | .09 | .10 | .09 | .93 | | |
| Crop IV: | | | | | | | |
| Percentage of N insoluble in acetic acid | 2.05 | 1.97 | 1.98 | 2.12 | 2.03 | 17.5 | 8.2 |
| Percentage of S soluble in acetic acid | .19 | .09 | .16 | .18 | ...... | | |
| Percentage of S insoluble in acetic acid | .11 | .13 | .11 | .11 | .115 | | |
| Inoculated series, Medford loam: | | | | | | | |
| Percentage of N insoluble in acetic acid | ...... | ...... | ...... | ...... | 2.20 | 17.6 | 11.0 |
| Percentage of S insoluble in acetic acid | ...... | ...... | ...... | ...... | .125 | | |
| Uninoculated series, Medford loam: | | | | | | | |
| Percentage of N insoluble in acetic acid | ...... | ...... | ...... | ...... | 1.21 | 20.0 | ...... |
| Percentage of S insoluble in acetic acid | ...... | ...... | ...... | ...... | .11 | | |

To say that percentage of sulphur will not increase regardless of sulphate concentration in the nutrient media without increasing the available nitrogen would not be in accordance with data already obtained. It does appear, though, that when the lack of nitrogen is sufficient to lower the nitrogen content compared to the normal nitrogen content of the clover there is a tendency toward decreased sulphur assimilation. It is interesting to compare the rape plant with the clover in this respect.

The figures given in Table IV show that the clover cut before the blossoming stage not only contains a higher percentage of total nitrogen but also a higher percentage of nitrogen insoluble in acetic acid. As the percentage of nitrogen removed by this fraction varies, so also does the percentage of sulphur. There appears to be a definite relationship between the sulphur and nitrogen content in this insoluble portion, thus adding more significance to this fraction in regard to quality and perhaps representing the true protein of the clover hay. No difference in ash content caused by variation in fertilizer treatment was observed in the different pots. The ash content of the different crops did vary however, as is shown in Table IV.

## EXPERIMENTAL WORK WITH THE RAPE PLANT

The Medford loam soil used in the first part of this work was used in this experiment. After the clover roots were removed the soil was returned to the pots and seeded to rape. Three gm. of sodium nitrate were added to those cultures which had received nitrate nitrogen in the clover experiment. The plants were harvested after a growing period of 50 days. At the end of this time there had been a cessation of growth, and the basal leaves dried up and fell off. The results appear in Table V.

TABLE V.—*Analytical results obtained with rape*

| Treatment. | Number of plants | Weight | Total N. | N insoluble in acetic acid. | Total S. | Sulphate S. | Organic S. | Ash. |
|---|---|---|---|---|---|---|---|---|
| | | *Gm.* | *Per cent.* | *Per cent* | *Per cent.* | *Per cent.* | *Per cent* | *Per cent.* |
| Control | [a] 7 | 12.0 | 1.93 | 0.90 | 0.35 | 0.16 | 0.19 | 13.6 |
| | 7 | 5.0 | 2.88 | 1.02 | .39 | .12 | .27 | 19.3 |
| $NaNO_3$ | 7 | 12.2 | 2.35 | .93 | .22 | .03 | .19 | 15.9 |
| | [a] 7 | 10.1 | 4.14 | .95 | .16 | .02 | .14 | 18.0 |
| $NaNO_3$ and $Na_2SO_4$ | 5 | 11.3 | 3.36 | .71 | 1.46 | 1.09 | .37 | 18.3 |
| | [a] 6 | 14.0 | 2.72 | .88 | 1.38 | .97 | .41 | 17.7 |
| $Na_2SO_4$ | 7 | 2.4 | 2.57 | .91 | 3.13 | 2.76 | .37 | 22.0 |
| | [a] 8 | 3.0 | 2.59 | ..... | 2.41 | 2.05 | .36 | 19.4 |
| $NaNO_3$ and $CaSO_4$ | [a] 6 | 14.45 | 2.30 | .83 | 1.26 | .83 | .43 | 14.7 |
| | 6 | 16.35 | 2.27 | .66 | 1.31 | .93 | .38 | 16.2 |
| $CaSO_4$ | [a] 7 | 2.4 | 2.60 | .88 | 3.28 | 2.98 | .30 | 21.9 |
| | 8 | 3.8 | 2.66 | ..... | 2.70 | 2.37 | .33 | 21.9 |

[a] Pots from the inoculated series.

The presence of added sulphates appeared to have retarded growth of the rape, as there is a greater dry-weight yield on the controls. With

the cultures receiving nitrate fertilizer the addition of sulphate sulphur apparently caused increased growth or had just the opposite effect. If the concentration of the sulphates was great enough to produce a toxic effect, the sodium nitrate may have counteracted this action.

The writer has observed just the opposite effect with clover seedlings growing on agar agar-mineral salt nutritive media. One gm. of sodium nitrate per liter had a noticeably toxic effect, while the same concentration of sodium sulphate produced no noticeably injurious effect. In the cultures containing both the same concentration of sodium nitrate and sodium sulphate there was an improvement in growth over the former sodium-nitrate cultures.

Application of nitrates produced very good yields on a comparative low sulphur assimilation by the plants. The question naturally arises whether the rape does not absorb sulphur, if present, far in excess of that required for carrying on the synthesis of its organic compounds. This appears so noticeable in comparing the figures in Table V. Of course it is realized that the optimum concentration of nutrients for plant nutrition has always been a problem. The acetic acid-insoluble nitrogen is higher in the rape grown on the soil receiving nitrate fertilizer only, compared with that in the rape which received both nitrate and sulphate fertilizer. There seems to be a tendency of the sulphates to decrease this form of nitrogen. Sulphate application increased the organic sulphur and total sulphur content of the rape, while at the same time the presence of these sulphate compounds retarded growth where no nitrates were added. The extremely high sulphate content is very obvious in these samples of rape. This may account for the high ash content. The percentage of ash in the samples of rape varies considerably, depending upon the fertilizer treatment and magnitude of growth. Such a variation did not occur with the clover.

## SUMMARY

Sodium sulphate and calcium sulphate had a beneficial effect on nodule development and nitrogen assimilation of the red clover grown on previously sterilized soil. On a similar series which was artificially inoculated with *Bacillus radicicola* at the time of seeding, sulphates caused no increase in nodule development.

When a soil of high sulphur content was used, the nitrogen content in clover of the third and fourth crops was lower on the control pots than where either sulphur, calcium sulphate, or sodium sulphate was applied. As sulphate sulphur was present in all plants, the low nitrogen content could not be explained by a cessation in protein synthesis due to the absence of sulphates.

This again shows the relation of sulphates to nitrogen assimilation and the favorable influence of sulphates on the legume bacteria or on some other agency controlling nitrogen assimilation.

The ratio of nitrogen to sulphur in the portion of the clover plant insoluble in dilute acetic acid remains about the same, regardless of the stage in the development of the plant. This gives further support to the view that the nitrogen insoluble in acetic acid represents protein nitrogen. The total nitrogen and total nitrogen insoluble in acetic acid was higher in those plants cut before the blossoming stage.

With clover growing on sand cultures, it was possible, by reducing the available nitrate, not only to limit the growth and nitrogen content but also to decrease the sulphur assimilation. So, while sulphates apparently cause greater nitrogen assimilation through their beneficial effect on nodule development, the amount of sulphur taken up by the plant is limited by the total nitrogen absorbed.

The rape plant assimilated a large amount of sulphur, although the presence of sulphates reduced the yield compared to the control soil cultures. Sulphate plus nitrate caused increased yields compared with those secured when nitrate was added alone. There does not appear to be any direct relation between nitrogen and sulphur assimilation in the rape plant.

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# SOYBEAN MOSAIC[1]

By MAX W. GARDNER, *Associate in Botany*, and JAMES B. KENDRICK, *Assistant in Botany, Purdue University Agricultural Experiment Station*

In a small field of Hollybrook soybeans in West La Fayette a typical mosaic disease was found August 25, 1920. A rather low percentage of the plants were affected, and the disease was more or less confined to one quarter of the field adjacent to which were several rows of garden beans affected with mosaic to a considerable degree. In another larger field of soybeans in the same locality no mosaic was found. Leafhoppers were very prevalent on the soybeans. The impression was gained that the disease might have spread from the garden beans to the soybeans, but as yet no evidence to support such a theory has been obtained.

Clinton[2] found soybean mosaic in 1915 at Mount Carmel, Conn., and under the name of chlorosis or crinkling has given an excellent account of the leaf symptoms along with a good illustration. He found the disease on the varieties Medium Green, Wilson, Swan, Kentucky, Wing's Mikado, and Hollybrook, and states that the Hollybrook showed the most marked symptoms. He found the chlorosis without the crinkling on the varieties O'Kute, Ito San, and Manhattan. C. R. Orton[3] has reported the occurrence of mosaic in a field of Ito San soybeans at Girard, Pa., July 30, 1920.

## SYMPTOMS

The mosaic symptoms on the soybeans were conspicuous and unmistakable, resembling those characteristic of mosaic diseases in general. Affected plants were stunted, and petioles and internodes were shortened to some extent. The leaflets were stunted, greatly misshapen, and puckered with dark-green puffy areas along the veins (Pl. 18, A, C, D, E). Between these puffy areas the leaf tissue was etiolated. Affected leaflets tended to be asymmetrical, twisted, and curled downward about the margins (Pl. 18, D, E). As in other mosaic diseases, the young, rapidly growing leaves showed the most severe effects, and in some cases whole leaflets or portions thereof were extremely stunted or killed outright by the disease (Pl. 18, B). The mosaic symptoms were readily distinguishable from a uniform crinkling of the leaflets which was rather common in this field and apparently attributable to insect injury.

The pods on mosaic plants were stunted and flattened, less pubescent, and more acutely curved than those on normal plants (Pl. 19, C, D).

[1] Contribution from the Botanical Department of Purdue University Agricultural Experiment Station, La Fayette, Ind.

[2] CLINTON, G. P. NOTES ON PLANT DISEASES OF CONNECTICUT. *In* Conn. State Agr. Exp. Sta. Ann. Rpt., 1915, p. 446–447, pl. 238. 1916.

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Oct. 8, 1921
Key No. Ind.–10
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Those borne at the upper nodes were more severely affected. The yield of seed was very materially reduced (Pl. 19, A, B), since a considerable proportion of the pods contained no germinable seeds and the remainder as a rule not more than one or two seeds (Pl. 19, D). Even the germinable seeds were in general undersized.

Observations made a month later showed that the mosaic plants were remaining green longer than the normal plants, so the disease evidently delayed maturity.

## FIELD INOCULATIONS

In another field of soybeans in which no mosaic was present inoculations were made August 27 by rubbing the young internodes with cotton soaked in the juice from crushed mosaic soybean leaves and then wounding these internodes with a needle. One hundred and fourteen plants were thus inoculated, but no mosaic developed. Fifty-two plants were similarly inoculated, except that the juice of leaves from mosaic garden beans was used as inoculum, and none developed the disease. Forty-six garden bean plants were also inoculated in a similar manner with the virus from soybean mosaic, and none developed mosaic.

## SEED TRANSMISSION

To determine whether or not the disease was seed-borne, a quantity of seed was saved from mosaic and healthy plants early in October for subsequent tests in the greenhouse. On October 25, 150 seeds from mosaic plants were planted in 25 pots of sterilized soil, 6 in each pot. By December 15, 124 plants had come up, and 18 showed unmistakable mosaic symptoms. None of the 148 controls grown from seed from normal plants showed mosaic.

In a second trial about 180 seeds from mosaic plants were planted December 9 in 59 pots of sterilized soil. February 3, 1921, 11 out of the 106 plants which were up showed mosaic. None of the 38 controls grown from seed from normal plants showed the disease. As a result of these two tests it is evident that about 13 per cent of the seedlings from seed produced on mosaic plants developed the disease.

The mosaic seedlings were spindling (Pl. 18, F, G), and the first pair of true leaves were characterized by downward, longitudinal curling or rolling, a crinkling, and a faint etiolation or mottling. These leaves turned yellow prematurely. The leaves subsequently formed were greatly stunted and showed the mottling and crinkling more conspicuously than the first leaves.

## GREENHOUSE INOCULATIONS

From these mosaic seedlings the disease was transmitted to healthy soybean seedlings. Several methods of inoculation proved successful. A number of inoculations made early in January yielded only negative results, but later better success was obtained.

On January 26, twenty-five plants were inoculated by pricking with a needle at the nodes and rubbing the wounded areas with cotton soaked in the juice from crushed mosaic leaves. Eight plants used as controls were similarly treated, except that sterile water was substituted for the mosaic virus. Because of the unfavorable greenhouse conditions the plants made slow growth during the winter, so that the mosaic symptoms were very slow in developing. On March 5 two plants showed mosaic mottling on the young leaves. On March 25 two more showed mosaic, and on April 7 seven out of the 25 plants had developed the disease. The controls developed no mosaic.

A number of inoculations were made March 2. In one series crushed mosaic tissue was inserted into slits made with a scalpel near the growing points and on the petioles. On March 15 two of the seven plants thus inoculated showed mosaic symptoms on the young leaves, and on April 7 five had developed mosaic.

In a second series of inoculations made the same date by cutting off one leaf at each node and smearing these wounded surfaces with crushed mosaic tissue, three out of eight plants showed mosaic symptoms on the new leaves March 15, or 13 days after inoculation, and on April 7 six plants had developed mosaic.

In a third series five plants were inoculated by a combination of the two methods above described. Thirteen days later three showed mosaic mottling, and by April 7, or 37 days after inoculation, four had developed the disease.

In a fourth series, five plants were inoculated by rubbing the under surfaces of the leaves with slightly crushed mosaic leaves forcibly enough to cause slight abrasions. On March 15, four of these plants showed the disease, and on April 7 all showed typical mosaic.

None of the five control plants inoculated by one or the other of these methods without the application of mosaic tissue developed mosaic. At no time was there any spread of the disease in the greenhouse.

In these inoculations the symptoms became evident only on the young leaflets. These in some cases developed distinct mottling, and in other cases they exhibited a slight degree of etiolation and the characteristic downward, longitudinal rolling. The incubation period under the conditions of this test was 13 days.

Preliminary cross inoculations to garden beans and cowpeas have given negative results. Further tests are being made.

Soybeans, therefore, are subject to a destructive mosaic disease which greatly reduces the yield of affected plants. The disease is transmissible from plant to plant and also is seed-borne.

PLATE 18

A.—Typical mosaic leaf showing darker green puffy areas along the veins.

B.—Mosaic leaf showing extreme stunting of terminal leaflet.

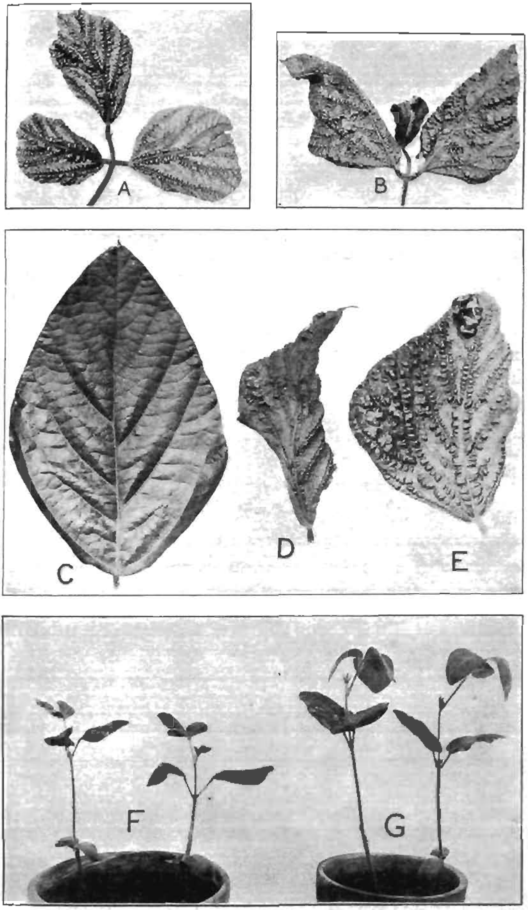
C.—Normal leaflet.

D.—Mosaic leaflet showing longitudinal rolling.

E.—Typical mosaic leaflet.

F.—Mosaic seedlings from seed from a mosaic plant, showing stunting of the plant and longitudinal rolling of first leaves.

G.—Normal seedlings from seed from a mosaic plant.


A
B
C
D
E
F
G

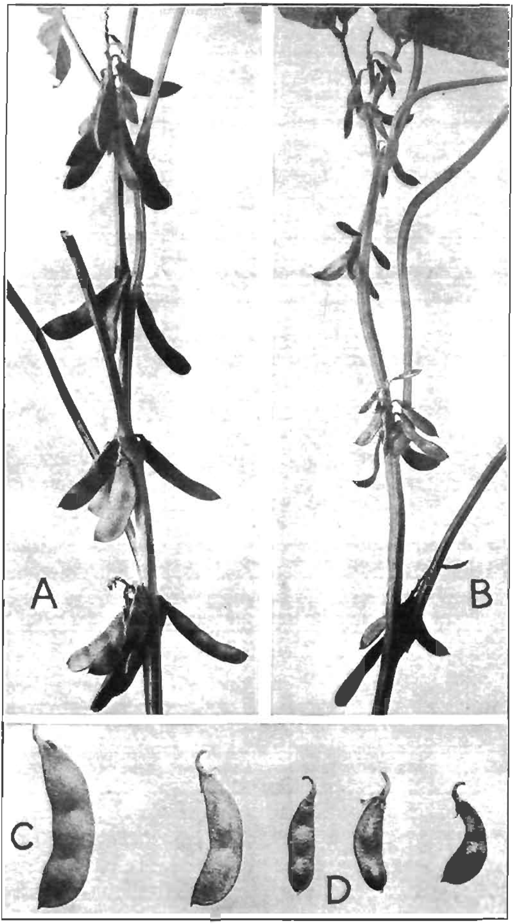

A
B
C
D

PLATE 19

A.—Upper nodes of a normal plant, showing yield of pods.
B.—Upper nodes of a mosaic plant, showing effect of the disease on the yield.
C.—Normal pod.
D.—Type of pods produced by a mosaic plant.

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# JOURNAL OF AGRICULTURAL RESEARCH

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## INFLUENCE OF THE PLANE OF NUTRITION ON THE MAINTENANCE REQUIREMENT OF CATTLE[1]


By F. B. MUMFORD, *Dean of the College of Agriculture*, A. G. HOGAN, *of the Department of Animal Husbandry*, and W. D. SALMON, *Graduate Student in Animal Husbandry, College of Agriculture, University of Missouri*.


In 1914 an investigation was begun at the University of Missouri to study some of the effects of underfeeding. Calves of beef-breeding stock were secured, and they were placed on three planes of nutrition. Group I was fed to grow rapidly, but not to become fat. Group II was placed on a lower nutritive plane and was fed to gain about ½ pound per day. Group III was placed on a still lower nutritive plane and fed to gain about ⅓ pound per day. At the present time three animals remain that were started on the investigation in 1914. Seven others were added in 1917. The older animals, therefore, have been under observation for seven years and the younger animals for four years. Under these circumstances it seemed desirable to make a study of the maintenance requirement of steers at different ages and on different planes of nutrition.[2]

The ideal method of conducting an investigation of this kind would require a respiration calorimeter. Since that was impossible, the alternative was to calculate the energy value of the feed consumed and correct this for the estimated value of the gains or losses in body weight.

The net energy of the feed consumed was calculated in accordance with procedures developed by Armsby.[3] The energy value of the changes in body weight were calculated from the composition of steers that had been analyzed at this Station by the Department of Agricultural Chemistry. So far as possible steers were selected as controls for this purpose that were of similar age, measurements, and weight and that had received similar treatment.

[1] The data for this paper were taken from the thesis of W. D. Salmon, presented at the University of Missouri, as partial fulfillment of the requirements for the degree of Master of Arts. The investigation was initiated by F. B. Mumford, Dean of the College of Agriculture, and by P. F. Trowbridge, at that time Chairman of the Department of Agricultural Chemistry. Since September 1918, E. A. Trowbridge, Chairman of the Department of Animal Husbandry, has had general supervision of the project. This article was prepared by A. G. Hogan, who has been in immediate charge since September, 1920. A large number of workers have contributed to the success of the experiment.

[2] The original data will be reproduced in detail in a subsequent publication.

[3] ARMSBY, H. P., and FRIES, J. A. NET ENERGY VALUES FOR RUMINANTS. Pa. Agr. Exp. Sta. Bul. 142, 20 p. 1916.

## METHOD OF THE EXPERIMENT

### RATIONS

The concentrate consisted of the following mixture: Corn chop, 60 per cent; wheat bran, 30 per cent; linseed meal, 10 per cent. The roughage fed to the 3 old steers, No. 528, 589, and 585, from the beginning of the experiment until July 20, 1917, was timothy. For the next 10 days a mixture of 5 parts timothy, 3 parts alfalfa, and 2 parts oat straw was fed. Following this the roughage consisted of a mixture of 60 per cent alfalfa amd 40 per cent oat straw. The animals were fed twice daily and had access to water at all times. Salt was accessible at feeding time.

### PERIODS

The calculations are made for periods of 180 days, with the exception of the first period for the 3 older steers, which was as follows: No. 528, 130 days; No. 579, 142 days; No. 585, 150 days. The warm months of the year were selected for these periods to avoid a possible disturbing effect of low temperatures in the winter months.

### WEIGHTS

The steers were weighed each morning after feeding but before watering. The weight given for the beginning of a period is the average of the 10 preceding days. The weight given at the end is an average of the last 10 days of the period

### ENERGY INTAKE

The amount of dry matter consumed was calculated from the weight and composition of the feed consumed. The net energy was computed from this by the use of factors reported by Armsby and Fries.[1] For the concentrates the value 83.82 therms per 100 pounds dry matter was used. This is the factor given for Armsby's grain mixture No. 2,[2] which approximates the grain mixture used in this experiment. For timothy hay the value 48.63 therms per 100 pounds dry matter was used. The factor for the roughage mixture used in the latter part of the experiment was calculated from the Armsby values, for alfalfa, 34.10 therms and for oat straw, 26.03 therms per 100 pounds dry matter. A mixture of 60 parts alfalfa and 40 parts oat straw would have a value of 30.87 therms per 100 pounds dry matter. The calculations of the energy value of the milk are based on factors published by Armsby.[3] There are 29.01 therms per 100 pounds whole milk (4.4 per cent) and 14.31 therms per 100 pounds skim milk (0.2 per cent). From these values factors were computed for the different grades of milk used.

[1] ARMSBY, H. P., and FRIES, J. A. OP. CIT.

[2] Armsby's grain mixture No. 2, 60 per cent corn meal, 30 per cent crushed oats, 10 per cent O. P. linseed meal. Our grain mixture, 60 per cent corn meal, 30 per cent wheat bran, 10 per cent O. P. linseed meal.

[3] ARMSBY, Henry Prentiss. THE NUTRITION OF FARM ANIMALS, p. 719. New York, 1917.

## CHANGES IN BODY WEIGHT

In order to obtain data concerning the maintenance requirement of these steers, it is necessary to calculate the energy gained or lost through changes in body weight. Our calculations are based on analyses previously made by the Department of Agricultural Chemistry, University of Missouri.[1] Control animals were selected from those on which analyses were available, on the basis of similar weights and measurements, and when possible of similar ages, daily gains, and daily consumption of dry matter. In some cases suitable control animals were not available, and the composition of steers for those periods was estimated by interpolation, with the exception of the last period for steer No. 528. In this case a value published by Armsby[2] was used. The average energy values of a pound gain as calculated by this method for steers in the three groups are given in Table I. For purposes of comparison the values given by Armsby are shown in the same table.

TABLE I.—*Energy values of a pound gain*

| Approximate age (months). | Group I. | Group II. | Group III. | Armsby's values | |
|---|---|---|---|---|---|
| | | | | Age. | Energy. |
| | *Therms.* | *Therms.* | *Therms.* | *Months.* | *Therms.* |
| 6 | 0.95575 | 0.95575 | 0.8343 | 1 | 1.170 |
| 18 | 1.0918 | 1.0583 | .9445 | 2 to 3 | 1.374 |
| 30 | 1.7136 | 1.1608 | 1.0548 | 5 to 6 | 1.680 |
| 54 | 2.1993 | 1.4104 | 1.1013 | 11 to 12 | 2.292 |
| 66 | 2.50 | 1.5352 | 1.4790 | 18 to 24 | 3.000 |
| 78 | 3.00 | 1.660 | 1.6490 | | |

Armsby's values are consistently higher, as is to be expected. Our animals were thin and contained less than the usual amount of fat in the gain.

In calculating the maintenance requirements per 1,000 pounds live weight, Moulton's[3] formula was used. He has shown that the surface areas of thin cattle are proportional to the 5/8 power of the live weight. The results of this calculation, on the basis of dry matter consumed, are given in Table II.

The net energy required for maintenance was also calculated by another method,[4] based on the digestible organic matter of the feed.

The following factors are given for the metabolizable energy of digestible organic matter consumed: Roughage, 1.588 therms per pound; grains and similar feeds with less than 5 per cent digestible fat, 1.769 therms per pound. In the same publication the "Average energy expenditure by cattle per 100 pounds of dry matter eaten" is given.

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[1] These have not yet been published

[2] ARMSBY, Henry Prentiss. OP. CIT.

[3] MOULTON, C. R. THE AVAILABILITY OF THE ENERGY OF FOOD FOR GROWTH In Jour. Biol. Chem., v. 31, no. 2, p. 390. 1917.

[4] ARMSBY, H. P., and FRIES, J. A. OP. CIT.

TABLE II.—*Average daily maintenance requirement as calculated from dry matter consumed*

| Steer No. | Number of periods averaged. | Therms of net energy per 1,000 pounds, based on ⅔ power of live weight. | | |
|---|---|---|---|---|
| | | Group I. | Group II. | Group III. |
| 528 | 6 | 5.870 | | |
| 577 | 3 | 5.280 | | |
| 571 | 3 | 5.730 | | |
| 579 | 5 | | 4.920 | |
| 578 | 3 | | 3.830 | |
| 573 | 3 | | 4.409 | |
| 585 | 5 | | | 4.221 |
| 575 | 3 | | | 4.041 |
| 574 | 3 | | | 4.302 |
| 572 | 3 | | | 3.250 |
| Average of all animals for all periods | | 5.523 | 4.485 | 3.830 |

TABLE III.—*Energy expenditure by cattle per 100 pounds dry matter consumed*

| Ration | Energy expenditure. |
|---|---|
| Roughage. | *Therms.* |
| Timothy hay | 35.47 |
| Alfalfa hay | 53.03 |
| Oat straw | 46.00 |
| Concentrate. | |
| Grain mixture No. 2 | 51.76 |

The coefficients of digestibility used in these calculations were derived from digestion trials conducted under similar conditions at this Station. These indicated that the digestibility of the ration varied with the relative amounts of hay and grain fed. The factors used are given in Table IV.

TABLE IV.—*Digestion factors for organic matter*

| Ratio of grain to hay | 1.1 | 2.3 | 1.2 | 1.3, 4, or 5 | 1.6 or 7 | 1.8, 9, or 10 | Hay only. |
|---|---|---|---|---|---|---|---|
| Factor | .6956 | .6695 | .6434 | .6340 | .6229 | .6030 | 0.5832 |

Inasmuch as the thermal value of a pound of organic matter from grain differs from that of a similar weight of organic matter from roughage, the Armsby factors[1] previously quoted in this paper could not be directly applied to the values obtained with the foregoing digestion coefficients. Those factors would not provide for the widely varying proportions of grain and hay. The following method, therefore, was

[1] ARMSBY, H. P., and FRIES, J. A. OP. CIT.

used in computing the energy intake on the basis of digestible organic matter consumed. By use of the factors in Table IV, the weight in pounds of digestible organic matter in the mixed ration was determined for each period. This was multiplied by 1.588, the Armsby factor for metabolizable energy in a pound of digestible organic matter from hay. The thermal value of digestible organic matter from grain is 1.769, however, or 0.181 therms more. Therefore, each pound of digestible organic matter derived from grain was multiplied by 0.181, and the product was added to the result obtained by multiplying the total digestible organic matter by 1.588. This gave the total metabolizable energy in both the hay and grain. The digestibility of the organic matter of the grain was estimated by difference. This ranged closely around 80 per cent. The factors for energy expenditure are given in Table III.

It seemed impracticable to calculate the net energy of the milk consumed on the basis of digestible organic matter, so the calculation was based on the quantity consumed, as previously described. Since the amount was small, however, the method of calculation would have little effect on the final result.

The method used in correcting for changes in body weight has already been described, and the maintenance requirement as calculated on the basis of digestible organic matter consumed is given in Table V.

TABLE V.—*Average daily maintenance requirement, as calculated from digestible organic matter consumed*

| Steer No. | Number of periods averaged. | Therms of net energy per 1,000 pounds based on 5/8 power of live weight. | | |
|---|---|---|---|---|
| | | Group I. | Group II | Group III. |
| 528 | 6 | 6.261 | | |
| 577 | 3 | 5.412 | | |
| 571 | 3 | 5.174 | | |
| 579 | 5 | | 5.260 | |
| 578 | 3 | | 4.192 | |
| 573 | 3 | | 4.893 | |
| 585 | 5 | | | 4.725 |
| 575 | 3 | | | 4.454 |
| 574 | 3 | | | 4.591 |
| 572 | 3 | | | 3.649 |
| | Average of all animals for all periods | 5.777 | 4.869 | 4.408 |

In determining the maintenance requirement on the basis of digestible organic matter, the calculations were based on digestion coefficients obtained at this Station under similar conditions. This method is probably more accurate than that of calculation on the basis of dry matter consumed, and for the animals concerned it gives a result about 10 per cent higher.

In calculating average results, obtained by both methods, four periods in which there were losses in live weight were omitted. The results for those periods were low, and we were uncertain as to whether the result was approximately correct or whether it was due to an incorrect assumption as to the energy value of the loss in weight. Most of the dry matter of the loss was probably fat, and if so, our calculation of its energy value was too low and so made our calculation of the maintenance requirement too low.

One steer, No. 585, had a navel infection during the first period, accompanied by a very high maintenance requirement. This period also was discarded in calculating averages.

## INFLUENCE OF NUTRITIONAL PLANE

There is a close parallel between the intake of net energy and the maintenance requirement of the animal. The record of steer 574 illustrates that tendency. For the first period the average daily intake of net energy was 3.884 therms per 1,000 pounds, based on the 5/8 power of the live weight; and the maintenance requirement was 3.818 therms. For the second period the energy intake was increased to 5.783 therms, and the maintenance requirement increased to 5.119 therms. In the third period the energy intake was 5.253 therms, and the maintenance requirement was 4.836 therms.

TABLE VI.—*Daily maintenance requirements of cattle—Net energy*

| Number of experiments. | Investigator. | Condition of animal. | Therms per 1,000 pounds live weight. | | |
|---|---|---|---|---|---|
| | | | Maximum. | Minimum. | Average. |
| | RESPIRATION EXPERIMENTS | | | | |
| 22 | Armsby and Fries [1] | Medium | 7.430 | 4.723 | 5.995 |
| 7 | Kellner [1] | do | 6.780 | 4.921 | 5.742 |
| | do | Fat | 8.871 | 7.319 | 7.946 |
| | LIVE-WEIGHT EXPERIMENTS | | | | |
| 10 | Armsby [1] | Thin | 7.044 | 6.136 | 6.505 |
| 3 | do | do | 6.039 | 4.713 | 5.423 |
| 6 | Haecker [1] | Medium | 5.676 | 4.662 | 5.021 |
| 3 | Evvard [1] | do | 7.850 | 6.450 | 7.180 |
| 7 | Eckles [1] | do | 7.079 | 5.841 | 6.173 |
| 1 | Shirky [2] | do [3] | | | 7.732 |
| 2 | do | Thin [4] | 5.0959 | 4.953 | 5.0245 |
| 3 | Our results | Group I | 7.380 | 4.915 | 5.777 |
| 3 | do | Group II | 5.724 | 3.809 | 4.869 |
| 4 | do | Group III | 5.217 | 3.276 | 4.408 |

[1] ARMSBY, Henry Prentiss. OP. CIT., p. 291.
[2] SHIRKEY, S. B. EXTENT TO WHICH GROWTH RETARDED DURING THE EARLY LIFE OF THE BEEF ANIMAL CAN BE LATER REGAINED. Univ. of Mo. thesis, 1919. (Unpublished.)
[3] Corresponds to group I of this experiment.
[4] Corresponds to group II of this experiment.

In comparing the maintenance requirements of the three groups it should be kept in mind that group I does not represent a high plane of nutrition. The aim was to secure maximum growth with no considerable fattening. Their maintenance requirements as computed in this paper correspond closely to the average of 22 respiration experiments by Armsby and Fries[1] and of 7 by Kellner,[1] on cattle in medium condition. A comparison of our results, and of those obtained by other investigations, is given in Table VI.

## INFLUENCE OF AGE

The ages represented in this experiment vary from 30 days for some of the calves at the beginning of the first period to more than 6 years at the close of the seventh period. Apparently there was no relation between the age and the maintenance requirement of these animals. Some of the steers showed a gradual decrease in the maintenance cost from the beginning to the end of the experiment. In such cases it was found that the energy intake per 1,000 pounds had also decreased. On the other hand, steers with an increasing energy intake showed an increased maintenance requirement. Maintenance trials on young animals usually give higher results than have been obtained with mature animals, but if age does influence the maintenance requirement the effect is too slight to be shown in a live-weight experiment of this kind.

## SUMMARY AND DISCUSSION

There is a close relation between the amount of net energy consumed and the maintenance requirement. Periods of high energy intake were apparently periods of high maintenance cost, while periods of low energy intake were accompanied by a lowered maintenance requirement.

The averages of the periods discussed show the following daily maintenance requirements per 1,000 pounds live weight, calculated on the basis of digestible organic matter, and in terms of net energy: Group I, 5.777 therms; group II, 4.869 therms; and group III, 4.408 therms. If the maintenance requirement of group I is 100 per cent, that of group II is 84.4 per cent, and that of group III is 76.3 per cent.

The calculations on the basis of dry matter consumed indicate even greater differences. The maintenance requirements as derived by this method may be compared as follows: Group I, 100 per cent; group II, 81 per cent; group III, 69.3 per cent. The estimated maintenance requirement of group I, as calculated by this method, is 30 per cent greater than the total net energy intake of group III.

There is no apparent relation between the age of the animals and the amount of energy required for maintenance.

[1] ARMSBY, Henry Prentiss. OP. CIT.

# TURNIP MOSAIC[1]

By MAX W. GARDNER, *Associate in Botany*, and JAMES B. KENDRICK, *Assistant in Botany, Purdue University Agricultural Experiment Station*

In one corner of a small field of turnips near South Bend, Ind., October 12, 1920, a considerable percentage of the plants were found affected with an unmistakable mosaic disease. The symptoms were typical of mosaic diseases in general. The leaves were stunted, misshapen, and a lighter green with dark green blisters or puffy areas. Many of the leaves were extremely distorted by crinkling and folding (Pl. 20, A). The disease seemed to be confined to one area in the field, to some extent coincident with a heavy infestation of tarnished plant bugs.

Several diseased plants were transplanted to pots in the greenhouse, where they continued to form new leaves during the winter. The mosaic symptoms exhibited by the new foliage formed under greenhouse conditions were not quite so extreme as had been noted in the field. One of these plants, with mottled and spindling leaves, is shown in Plate 20, B, as it appeared in December.

Inoculation of a number of potted turnip and radish seedlings was made by breaking off a leaf and rubbing the wound with crushed leaf tissue from one of the mosaic plants. Out of 21 turnip seedlings inoculated early in January, 13 developed characteristic mosaic symptoms. The first symptoms were noted 26 days after inoculation. The turnips inoculated showed some varietal difference from the plants collected in the field in that the leaves were much less distinctly pinnatifid. Out of 46 radish seedlings, including both white and red varieties, similarly inoculated, none developed mosaic symptoms.

A later series of inoculations was made January 26 by wounding the plants with a needle and rubbing the wounded areas with a piece of cotton soaked in the juice from mosaic leaves ground up in a mortar. Ten out of 14 turnip plants thus inoculated developed the mosaic disease. The first symptoms were noted 16 days after inoculation. No mosaic developed among 13 control plants similarly treated except that sterile water was substituted for the mosaic virus. Twenty-two radish plants were also inoculated, and none of these developed the disease. Subsequent reinoculation of turnip plants from one of these radish plants produced no mosaic. The mosaic disease of turnips is therefore readily transmissible to turnips but not to radishes.

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[1] Contribution from the Botanical Department of Purdue University Agricultural Experiment Station, LaFayette, Ind.

After this article was prepared it was learned that Eugene S. Schultz, of the Bureau of Plant Industry, United States Department of Agriculture, was also working on this disease.

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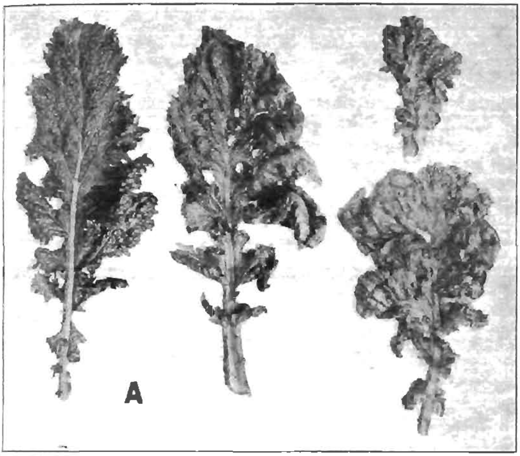
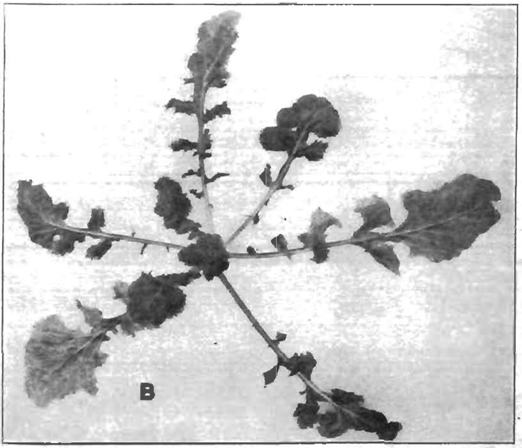
PLATE 20

A.—Leaves from mosaic turnip plants collected October 12, 1920.

B.—Mosaic turnip plant transplanted to a pot in the greenhouse. Photographed December 20, 1920.


A


B

# HYDROCYANIC ACID IN SUDAN GRASS[1]

By C. O. SWANSON[2]

*Professor of Agricultural Chemistry, Kansas State Agricultural College*

In a previous paper[3] it was shown that hydrocyanic acid (HCN) is obtained from green Sudan grass by macerating, digesting in water, and distilling into a dilute solution of sodium or potassium hydroxid. Several experiments reported in that paper made it clear that this acid does not exist free in Sudan grass and is obtained only if the conditions of the determination are favorable to enzym action. It appears to be a common belief that hydrocyanic acid is developed by freezing. This merely bursts the green cells and thus performs the same function as maceration, with the result that the hydrocyanic acid is rapidly lost from frosted grass. It was also shown that while in some cases poisoning had been reported from pasturing Sudan grass, under normal conditions no poisoning took place either before or after the grass was frozen. It was suggested that when frozen the hydrocyanic acid had been liberated and then evaporated as the grass dried.

Because of the importance of the subject it was thought worth while to make further investigations. During the summer of 1920, material was obtained from a 1/20-acre plot of Sudan grass grown by the Department of Agronomy of the Kansas State Agricultural College. The Sudan grass had been planted early in June in rows about 2 feet apart. On June 22, when the experiments were begun, the grass was about 6 inches high. These experiments were continued during the summer and early fall.

## METHOD OF DETERMINING HYDROCYANIC ACID

At present there are no satisfactory quantitative methods for estimating hydrocyanic acid obtained from organic material. All are open to some objection. After considering several, the Prussian-blue method was adopted as best suited for the purposes of the present investigation. Because of simplicity in manipulation it is possible to run a large number of determinations at the same time. The amount of hydrocyanic acid obtained from the different samples was estimated colorimetrically, using standard solutions containing known amounts of potassium cyanid. One objection to the colorimetric measurements was the difficulty in

[1] Contribution No. 92 from the Department of Chemistry, Agricultural Experiment Station of Kansas State Agricultural College.

[2] Credit is due Mr. Carl M. Conrad for efficient assistance in making the determinations reported in this paper.

[3] SWANSON, C. O. HYDROCYANIC ACID IN SUDAN GRASS AND ITS EFFECT ON CATTLE. *In* Jour. Amer. Soc. Agron., v. 13, no. 1, p. 33–36. 1921.

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obtaining a uniform blue color. Very often the precipitate was decidedly green. It was found that by warming and letting the precipitate stand for some time in loosely stoppered bottles a uniform blue color could be obtained. The use of nitric or sulphuric acid instead of hydrochloric acid or the addition of potassium fluorid, all of which have been suggested by other workers, did not seem to eliminate, entirely, the green color. While the defects of the Prussian-blue method are fully realized, it compares favorably with other methods.[1] In no sense are the values reported in this paper to be regarded with the same degree of accuracy as a protein or even a crude-fiber determination. For this reason no conclusions should be drawn from the results unless the figures presented are uniformly consistent or the differences large.

The calculations in this paper are based upon approximately 200 gm. of green material. When the grass was wilted or dry the weight of sample used was proportionately lessened. It is impracticable to secure green samples of uniform weights of dry matter, particularly if they are gathered during different hours of the day and throughout several weeks and months. Then, as will be shown in what follows, the hydrocyanic acid is localized in the plant, being present in the largest amounts in those portions of the plant possessing the greatest vegetative activity. For this reason leaves were separated from the stems whenever these were present. The amount of hydrocyanic acid obtained is small in proportion to the total weight of samples used. It was seldom more than 0.015 per cent.

## EFFECTS OF MACERATION

The first sample was collected June 22, when the grass was about 6 inches high. This was cut into pieces about ¼ inch long and digested for three hours in water at room temperature. Less than 1 mgm. hydrocyanic acid was obtained. Another sample, taken the next day, was cut and thoroughly macerated by pounding in an iron mortar and was then digested in water. This sample gave 27 mgm. hydrocyanic acid. On June 28 a sample was secured and divided into two equal portions. One portion was cut and macerated as described above, and the other was cut and macerated with coarse, sharp sand. Both were digested in water for the same length of time. The portion macerated with sand gave 26 mgm. hydrocyanic acid, and the other gave 36 mgm. It appeared from this that maceration with sand was not necessary and might result in a loss. Subsequent experiments showed that as soon as the grass is macerated the hydrocyanic acid is liberated and for this reason may be lost. On August 18 a sample was divided into four portions and, after the preliminary treatment mentioned, was digested overnight, with the result given in Table I.

[1] VIEHOVER, Arno, and JOHNS, Carl O. ON THE DETERMINATION OF SMALL QUANTITIES OF HYDROCYANIC ACID. *In* Jour. Amer. Chem. Soc., v. 37, no. 3, p. 601-607. 1915.

TABLE I.—*Effect of maceration on liberation of hydrocyanic acid*

| Sample No. | Treatment. | HCN. |
|---|---|---|
| | | *Mgm.* |
| 1 | No cutting or maceration | 0 |
| 2 | Cut in feed cutter (¼ to ⅛ inch) | 10 |
| 3 | Cut and macerated slightly | 10 |
| 4 | Cut and macerated thoroughly | 11 |

From this it appeared that if the time of digestion is sufficiently long the amount of maceration is less important, provided, however, that the plant tissue is cut fairly fine. The smaller amount obtained from the grass on August 18, as compared with that obtained in June, is in accord with a general observation made during the summer, that as the season advanced smaller amounts were obtained from the 200-gm. portions.

## TIME REQUIRED FOR DIGESTION

The time required for digestion in order to obtain the maximum amount of hydrocyanic acid was determened. A sample collected on June 28 was divided into three portions and similarly treated, except for the time allowed for digestion. The results obtained are given in Table II.

TABLE II.—*Effect of time of digestion on liberation of hydrocyanic acid*

| Sample No. | Treatment. | HCN. |
|---|---|---|
| | | *Mgm.* |
| 1 | Digested 3 hours | 18 |
| 2 | Digested 6 hours | 32 |
| 3 | Digested 24 hours | 32 |

This experiment seemed to show that digesting 3 hours was not long enough, while 6 hours was as effective as 24. On August 28 a similar experiment gave the results shown in Table III, the results in each case being an average of duplicate samples.

TABLE III.—*Effect of time of digestion on liberation of hydrocyanic acid*

| Sample No. | Treatment. | HCN. |
|---|---|---|
| | | *Mgm.* |
| 1 | Digested two days | 10 |
| 2 | Digested three days | 10 |
| 3 | Digested four days | 10 |
| 4 | Digested seven days | 0 |

The portion digested seven days developed a very bad odor. Because of these results, the usual procedure with experiments reported in this paper was to macerate the sample and then digest at room temperature overnight.

## LOCALIZATION OF HYDROCYANIC ACID IN THE PLANT

At three different times the grass was divided into leaves and stems. From 200-gm. portions the number of milligrams of hydrocyanic acid shown in Table IV were obtained.

TABLE IV.—*Hydrocyanic acid in leaves and stems of Sudan grass*

| Date collected | Leaves. | Stems. |
|---|---|---|
| | *Mgm.* | *Mgm.* |
| June 30 | 67 | 5 |
| July 7 | 33 | Trace. |
| July 21 | 66 | 0 |

The immaturity of the sample collected June 30 accounts for the moderate amount obtained from the stems. At a later date two tests were made on immature heads. No hydrocyanic acid was found. In the following tests reported in this paper leaves only were used unless otherwise stated.

## INFLUENCE OF STAGE OF GROWTH

Since grass was cut almost every week throughout the summer there were afforded several opportunities to test the comparative amounts present in various stages of growth and development. The shorter grass was obtained from plants which had been cut once or several times.

TABLE V.—*Hydrocyanic acid in Sudan grass at different dates and stages of growth*

| Date collected | Portion used | Average height. | HCN. |
|---|---|---|---|
| | | | *Mgm.* |
| July 22 | Whole plant | 6 inches | 27 |
| | do | 8 inches | 19 |
| | do | 12 inches | 7 |
| | Leaves | 18 inches | 24 |
| | do | 24 inches | 20 |
| | do | 30 inches | 9 |
| 26 | Whole plant | 4 inches | 40 |
| | do | 6 inches | 32 |
| | do | 12 inches | 10 |
| | Leaves | Beginning to head | 12 |
| | do | Partly headed | 17 |
| | do | Fully headed | 20 |
| | do | Partly dead | 6 |
| 31 | Whole plant | 4 inches | 11 |
| | Leaves | 16 inches | 18 |
| | do | Headed | 19 |
| Aug. 12 | Whole plant | 4 inches | 40 |
| | do | 10 inches | 12 |
| | Leaves | 15 inches | 16 |
| | do | 24 inches | 10 |
| 27 | Whole plant | 5 inches | 10 |
| | Leaves | 20 inches | 11 |
| 30 | Whole plant | 2 inches | 5 |
| | do | 12 inches | 5 |
| | Leaves | Ready to head | 9 |
| | do | Blooming | 10 |

The results show that more hydrocyanic acid is found in the whole plant in the earlier stages of growth and less as the season advances. The difference is perhaps due to the large proportion of stems in the latter part of the season, since if leaves only are compared there is very little difference except where they are from mature plants. This indicates that most of the hydrocyanic acid is obtained from those parts of the plants where the vegetative activity is most pronounced. This agrees with the results obtained by Menaul and Dowell [1] at the Oklahoma Agricultural Experiment Station. These observations support the theory that hydrocyanic acid is an intermediate product between the nitrates and the amino acids.[2]

## DISAPPEARANCE FROM MACERATED MATERIAL

As soon as the grass is macerated the hydrocyanic acid begins to pass off. This was demonstrated several times by suspending small pieces of sodium-picrate paper above some macerated grass in stoppered flasks. The paper very soon assumed a brown color. The quantitative determinations given in Table VI were made on samples macerated July 10 and treated as indicated.

TABLE VI.—*Disappearance of hydrocyanic acid in macerated grass*

| Sample No. | Treatment | HCN. |
|---|---|---|
| | | *Mgm.* |
| 1 | Digested in water two days | 25 |
| 2 | Placed without added water in covered mason jar for two days then small amount of water added and distilled | 12 |
| 3 | Placed in flask two days so that the hydrocyanic acid could escape only into the receiving flask, after which water was added and distilled | 26 |
| 4 | Left in open jar for two days, digested and distilled | Trace. |
| 5 | Repeat of 3 but kept in flask overnight only | 26 |

## DISAPPEARANCE FROM GRASS AFTER CUTTING

In a previous paper [3] it was stated that tests made on partially wilted grass may be worthless. In the experiments made at that time, the amount of sulphuric acid added was not carefully enough controlled. It will be shown in the following paragraphs that if acid is added beyond certain limits no hydrocyanic acid will be obtained from either green or partially wilted grass. In each of the determinations given in Table VII the grass was macerated after the treatment stated and then digested in water overnight.

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[1] MENAUL, Paul, and DOWELL, C. T. CYANOGENESIS IN SUDAN GRASS: A MODIFICATION OF THE FRANCIS-CONNELL METHOD OF DETERMINING HYDROCYANIC ACID. *In* Jour. Agr. Research, v. 18, no. 8, p. 447-450. 1920.

[2] RAVENNA, C., and ZAMORANI, M. NUOVE RICERCHE SULLA FUNZIONE FISOLOGICA DELL, ACIDO CIANIDRICO NEL SORGHUM VULGARE. *In* Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., v. 18, sem 2, no. 8, p. 283-287. 1909. Abstract *in* Chem. Abs., v. 5, no. 6, p. 1123. 1911.

[3] SWANSON, C. O. HYDROCYANIC ACID IN SUDAN GRASS AND ITS EFFECT ON CATTLE *In* Jour. Amer. Soc. Agron., v. 13, no. 1, p. 33-36. 1921.

TABLE VII.—*Disappearance of hydrocyanic acid from Sudan grass after cutting*

| Date collected | Sample No. | Treatment. | HCN. |
|---|---|---|---|
| | | | *Mgm.* |
| June 29 | 1 | Wilted in shade | 28 |
| | 2 | Green, control sample | 36 |
| July 7 | 3 | Wilted in sun for three hours | 12 |
| | 4 | Dried in sun from morning till evening, outdoors overnight | 15 |
| | 5 | Dried in shade for same length of time as 2 | 24 |
| | 6 | Dried outdoors two days and nights | 15 |
| | 7 | Dried in the shade two days and nights | 7 |
| | 8 | Dried in shade three days and nights | 20 |
| Aug. 12 | 9 | Dried in the shade five days | 32 |
| Sept. 1 | 10 | Dried in the shade two days | 6 |

While these results are not uniform, they do show conclusively that hydrocyanic acid can be obtained from wilted grass. Because of this result an attempt was made to determine more accurately the amount of hydrocyanic acid that may be obtained from wilted and dried grass.

## EFFECTS OF KEEPING GREEN GRASS MOIST AFTER IT IS CUT

A large sample of grass collected June 29 was placed stems down in a large bottle so that about one-fourth was immersed in water. At the end of different periods of time 200-gm. portions of the leaves were macerated and digested in water overnight. The amount of hydrocyanic acid obtained is given in Table VIII.

TABLE VIII.—*Effect of keeping grass moist after cutting*

| Sample No. | Length of treatment. | HCN. |
|---|---|---|
| | | *Mgm.* |
| 1 | 6 hours | 32 |
| 2 | 22 hours | 20 |
| 3 | 30 hours | 8 |
| 4 | 48 hours | 2 |

The results indicate that hydrocyanic acid slowly disappears from the grass after it is cut, but also that the grass may be kept for a while in the green condition without much loss of the hydrocyanic acid. Control samples taken at this time gave 30 mgm. hydrocyanic acid.

When the grass was wholly covered with water or when the air was excluded the results were different. In each case in the experiment reported in Table IX, unless otherwise stated, the grass was macerated and digested at the end of the treatment given.

This shows that the presence or absence of air has an intimate relation to the evolution of hydrocyanic acid. Experiments were performed in which the grass was kept in an atmosphere of carbon dioxid and also of

hydrogen. No hydrocyanic acid was obtained from the grass kept in an atmosphere of hydrogen, whereas from that kept in carbon dioxid considerable amounts were obtained. Some macerated grass was also placed in a desiccator from which the air was exhausted continuously. This did not seem to affect the amount of hydrocyanic acid obtained, but the experiment was not satisfactory. The effect of keeping the grass in different atmospheres needs further study.

TABLE IX.—*Effect of different treatments after cutting on hydrocyanic acid content*

| Date collected | Treatment. | HCN. |
|---|---|---|
| | | *Mgm.* |
| July 8 | Placed uncut in bottles and covered with water two days | ½ |
| | Distillate from this water | 1 |
| | Placed uncut in sealed mason jar with small amount of water two days | 1 |
| | Placed uncut in sealed mason jar with small amount of chloroform two days | 12 |
| | Placed uncut in sealed mason jar for 2 days, no water | 8 |
| | Placed uncut in bottle 2 days, covered with water | 2 |
| July 10 | Obtained by distilling water from this | 2 |
| | Macerated and digested 2 days in water | 25 |
| | Macerated and placed in bottle 2 days then water added and distilled | 12 |
| | Macerated and placed in open pan 2 days | Trace. |

## EFFECT OF HOT WATER

To determine this relation, enough grass was cut to make twenty-four 200-gm. portions of leaves. After the preliminary treatments as indicated in Table X, one set of 12 samples was digested in cold water and another set of 12 samples in hot water.

TABLE X.—*Effect of adding hot water on amount of hydrocyanic acid obtained*

| Time of drying in shade (hours). | Treatment before digestion. | Time of digestion. | HCN obtained after adding— Water at room temperature. | HCN obtained after adding— Boiling water |
|---|---|---|---|---|
| | | | *Mgm.* | *Mgm.* |
| 0 | Uncut | 5 hours | 0 | 0 |
| | Cut in feed cutter | do | 0 | 0 |
| | Macerated | do | 10 | Trace. |
| | Uncut | 24 hours | 7 | 0 |
| | Cut in feed cutter | do | 14 | 0 |
| | Macerated | do | 34 | 0 |
| 7 | Uncut | Overnight | 8 | 0 |
| | Cut in feed cutter | do | 12 | 1 |
| | Macerated | do | 19 | 1 |
| 28 | Uncut | 30 hours | 4 | Trace. |
| | Cut in feed cutter | do | 10 | 2 |
| | Macerated | do | 19 | 2 |

This shows that it is possible to obtain some hydrocyanic acid from the uncut green grass if the time of digestion is sufficiently long. In every case more was obtained when the material was cut in the feed cutter and still more when it was macerated. Hot water placed on the green material entirely prevented liberation. The small amount obtained from the partially wilted grass when the hot water was added was probably in a free condition at the time of adding the hot water. Almost as much hydrocyanic acid was obtained from the grass that was wilted seven hours as from the fresh grass if digested in water at room temperature sufficiently long.

This experiment as well as several others show that under some circumstances it is possible to obtain hydrocyanic acid from wilted or dried grass both with and without digestion in either hot or cold water. To investigate this further the following experiment was planned and executed. Five sets of 12 samples were secured and treated as follows: (1) Dried in the sun; (2) dried in the shade; (3) exposed in the sun, but kept moist by frequent sprinkling with water; (4) exposed in the shade but kept moist by sprinkling with water; (5) frozen in an ice machine and then exposed in open pans in the shade. The duration of these treatments was for 4, 8, 24, 31, and 48 hours, respectively. Six of the samples from each set were macerated after the period of the preliminary treatment, and hot water was poured on and distilled at once. The other six were digested in cold water overnight and then distilled. The results are shown in Table XI.

TABLE XI.—*Rate of disappearance of hydrocyanic acid from Sudan grass after it is cut and variously handled*[a]

| Time of treatment. | Hours of preliminary treatment. | Dried in sun, treated with— | | Exposed in sun but kept wet and treated with— | | Dried in shade and treated with— | | Exposed in shade but kept wet and treated with— | | Frozen before exposed in shade and treated with— | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hot water. | Cold water. | Hot water | Cold water | Hot water | Cold water. | Hot water. | Cold water | Hot water | Cold water |
| 9 a. m. first day | 0 | 0 | 16 | .... | . | 0 | 16 | 0 | 14 | 2 | 8 |
| 9 a. m. first day to 1 p. m. first day | 4 | 6 | 12 | Trace. | 4 | 4 | 14 | ... | .... | Trace. | 8 |
| 9 a. m. first day to 5 p. m. first day | 8 | 5 | 10 | 8 | 8 | 1 | 10 | .... | ...... | 0 | 6 |
| 9 a. m. first day to 9 a. m. second day | 24 | 6 | 10 | 1 | 4 | 2 | 0 | 9 | 5 | 1 | Trace. |
| 9 a. m. first day to 5 p. m. second day | 31 | ½ | 3 | Trace. | 6 | 6 | 5 | 8 | 8 | 0 | Trace. |
| 9 a. m. first day to 9 a. m. third day | 48 | Trace. | Trace. | 0 | Trace. | 5 | 7 | 4 | 10 | 0 | 10 |

[a] The figures indicate milligrams of hydrocyanic acid from 200 gm. of grass and are averages of several determinations.

The results show that no hydrocyanic acid is obtained from green material when treated with hot water very soon after cutting and macerating, but that when the grass is wilted as much as four hours in the sun, considerable hydrocyanic acid is obtained by treating with hot water immediately after maceration. The amount of hydrocyanic acid

obtained was not greater when the grass was wilted for a longer time. Less hydrocyanic acid is obtained from grass that is kept moist while in the sun than from grass that is allowed to dry rapidly. According to Ravenna and Zamorani [1] the nitrogen passes through the following stages in the plant: Nitrate → hydrocyanic acid → amino substance → protein substance. According to this theory the cells which continue to be active use the hydrocyanic acid for the building of protein substance, and as more nitrates from the soil are not supplied for manufacture of more hydrocyanic acid, the potential amount present when the plant is cut is soon exhausted.

When the grass was dried slowly in the shade the hydrocyanic acid disappeared more slowly than when it was dried in the sun, and the amount obtained from the hot-water treatment became approximately equal to that obtained from the longer digestion in cold water. This seems to mean that when the plant wilts the hydrocyanic acid is split off from glucocids and held in such loose combination that it can be set free by hot water and that practically all the hydrocyanic acid is in such combination, since additional amounts can not be obtained by further digestion. Splitting off begins as soon as the plant is cut. Determinations made on grass kept moist in the shade appear to show that after 24 hours all the hydrocyanic acid not otherwise used by the cells is in such a condition that it is soluble in water.

In the test in which the grass was frosted the hydrocyanic acid disappeared very rapidly, though the results were not very consistent.

## EFFECT OF ACIDS

On June 24 a sample of grass was placed in a flask after maceration, covered with water, and sulphuric acid was added to acid reaction. After it was digested and distilled as usual only a trace of hydrocyanic acid was obtained. From a sample of like material and similarly treated, except that no acid was added, 27 mgm. were obtained. On June 29 this experiment was repeated with the result that 8 mgm. were obtained when acid was used and 26 mgm. when it was not used. On June 30, 1 and 28 mgm. were obtained by these respective treatments. These experiments clearly indicate that the presence of acid has a very importance influence on the amount of hydrocyanic acid that may be obtained. To test the effect of the amount of acid used, four samples were prepared on July 14 and digested overnight in the following: (1) water; (2) *N/0.1* sulphuric acid ($H_2SO_4$); (3) *N/0.2* sulphuric acid; (4) *N/1* sulphuric acid. No hydrocyanic acid was obtained from any of the treatments with sulphuric acid, whereas the water digestion gave 30 mgm. On August 4 this experiment was repeated, using a weaker acid solution. Digestion in water gave 10

[1] RAVENNA, C., and ZAMORANI, M. NUOVE RICERCHE SULLA FUNZIONE FISIOLOGICA DELL' ACIDO CIANIDRICO NEL SORGHUM VULGARE. *In* Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., v 18, sem. 2, no. 8, p. 283-287. 1909. Abstract *in* Chem. Abs. v. 5, no. 6, p. 1123. 1911.

mgm. of hydrocyanic acid; *N*/0.01 sulphuric acid, 11 mgm.; and in *N*/0.02 sulphuric acid, 4 mgm. On August 2 three samples were prepared and digested in *N*/1 sulphuric acid; in *N*/0.2 sulphuric acid; and in *N*/0.05 sulphuric acid. Just before distillation, sodium hydroxid was added to almost neutral reaction. From the *N*/0.05 sulphuric acid 18 mgm. of hydrocyanic acid were obtained; a trace was obtained from the *N*/0.2, and none from normal. The weakest of the acid solutions gave no more than water alone. The smaller amounts obtained from the water treatments at the later date is in accord with the general observation that as the season advanced less hydrocyanic acid was present. It was planned to determine the exact hydrogen-ion concentration at which the hydrocyanic acid is most easily split off, but time did not permit. It is hoped that this may be determined in the future.

It was shown in connection with the hot-water treatment that when grass dries the hydrocyanic acid is changed into a free condition, so that simply adding hot water and distilling will drive off the hydrocyanic acid. To see if more would be driven off if acid was also present the following experiment was performed. Six samples of leaves were placed in the open in clear weather from 9 a. m. till 9 a. m. the next day. Then they were macerated and digested in water and in different concentrations of sulphuric acid. The results are given in Table XII.

TABLE XII.—*Effect of acid solutions in formation of hydrocyanic acid*

| $H_2SO_4$ added | HCN obtained. | $H_2SO_4$ added. | HCN obtained. |
|---|---|---|---|
| | *Mgm.* | | *Mgm.* |
| *N*/1 | 0 | *N*/0.02 | Trace. |
| *N*/0.2 | 0 | *N*/0.01 | 10 |
| *N*/0.05 | 0 | Water | 18 |

Thus, it appears that sulphuric acid is unfavorable to the liberation of the hydrocyanic acid even in the wilted material.

To determine whether hot sulphuric acid would liberate the hydrocyanic acid, hot water and sulphuric acid of varying normalities were added to green material immediately after maceration on July 16. The results are shown in Table XIII.

TABLE XIII.—*Effect of hot sulphuric acid on formation of hydrocyanic acid*

| $H_2SO_4$ added. | HCN obtained. | $H_2SO_4$ added. | HCN obtained. |
|---|---|---|---|
| | *Mgm.* | | *Mgm.* |
| *N*/1 | Trace. | *N*/0.05 | 0 |
| *N*/0.5 | 0 | *N*/0.02 | Trace. |
| *N*/0.2 | 0 | *N*/0.01 | Trace. |
| *N*/0.1 | Trace. | Hot water | 5 |

This shows that the use of hot acid is similar to that of hot water and that acid has no power to split off the hydrocyanic acid, at least in the concentration used. The traces obtained in some cases were no doubt due to liberation of hydrocyanic acid during maceration. It would, appear, however, that hot water was less destructive than hot acid. Like experiments with hydrochloric acid were performed with similar results.

On July 21 eight samples were prepared and digested at room temperature in phosporic acid (Table XIV).

TABLE XIV.—*Effect of phosphoric acid on liberation of hydrocyanic acid*

| $H_3PO_4$ added. | HCN obtained. | $H_3PO_4$ added. | HCN obtained. |
|---|---|---|---|
| | *Mgm.* | | *Mgm.* |
| *N/1* | 0 | *N/0.05* | 0 |
| *N/0.5* | 0 | *N/0.02* | 8 |
| *N/0.2* | 0 | *N/0.01* | 8 |
| *N/0.1* | 0 | Water | 8 |

The results indicate that the inhibiting power of phosphoric acid ($H_3PO_4$) was somewhat less than that of hydrochloric (HCl) or sulphuric acid. This would be expected since the degree of ionization of phosphoric acid is less than that of hydrochloric or sulphuric acid. Experiments with tartaric acid gave similar results.

## EFFECT OF DIGESTING IN ALKALINE SOLUTION

On July 20, 16 samples were prepared and digested in sodium-hydroxid (NaOH) and sodium-carbonate ($Na_2CO_3$) solutions, respectively (Table XV).

TABLE XV.—*Effect of alkaline solution on formation of hydrocyanic acid*

| NaOH added. | HCN obtained. | $Na_2CO_3$ added. | HCN obtained. |
|---|---|---|---|
| | *Mgm.* | | *Mgm.* |
| *N/1* | 0 | *N/1* | 0 |
| *N/0.5* | 0 | *N/0.5* | 0 |
| *N/0.2* | 0 | *N/0.2* | 0 |
| *N/0.1* | 0 | *N/0.1* | 0 |
| *N/0.05* | 0 | *N/0.05* | Trace. |
| *N/0.02* | 11 | *N/0.02* | 17 |
| *N/0.01* | 18 | *N/0.01* | 17 |
| Water | 19 | Water | 20 |

The results show the same general effect as that secured with acid solutions.

## EFFECT OF ACID OR ALKALI ON HYDROCYANIC ACID AFTER IT IS LIBERATED

An experiment was performed to show what effect acid or alkaline solutions have on the hydrocyanic acid after it is liberated. The green, macerated material was digested overnight in measured amounts of water. Enough standardized acid or alkali was then added to give the normality desired, and distilled. The results are given in Table XVI.

TABLE XVI.—*Effect of acid and alkali on hydrocyanic acid after it is liberated*

| Solution added | HCN obtained after treatment with solutions of— | | | | |
|---|---|---|---|---|---|
| | *N/1.* | *N/0.2.* | *N/0.1.* | *N/0.05.* | Water. |
| | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* | *Mgm.* |
| $H_2SO_4$ | 10 | 17 | ........ | 14 | 20 |
| HCl | 9 | 13 | 16 | 18 | 32 |
| $H_3PO_4$ | 16 | 8 | 20 | 18 | 32 |
| NaOH | 11 | 11 | 2 | 0 | 20 |

No hydrocyanic acid passed over in the first distillate from the sodium-hydroxid solution. The mixture was acidified with sulphuric acid and then distilled with the results given in Table XVI. While the results obtained in this experiment are not very uniform, they do show that hydrocyanic acid can be obtained from acid and alkaline solutions if the hydrocyanic acid is in a free condition before the acids are added. The experiment also appears to show that the addition of acid or alkali resulted in diminishing the amount of hydrocyanic acid obtained. The experiment was also tried by digesting the grass in sulphuric acid and sodium hydroxid of the normalities *N/1*, *N/0.2*, *N/0.1*, and *N/0.05* and then neutralizing before distilling. In no case was any hydrocyanic acid obtained.

## INFLUENCE OF WEATHER

From a sample taken June 23, when there had been no rain for three weeks, 27 mgm. of hydrocyanic acid were obtained. On June 30, after a heavy rain and a week of good growing weather, during which there was plenty of moisture, 30 mgm. were obtained. On July 24, when there had been a period of dry weather, the amount obtained was 7 mgm. The next day, following a rain during the night, the amount was 16 mgm. Several experiments indicated that the largest quantity was obtained when the plant was in the most vigorous growing condition. This is contrary to a common belief that stunting has some effect in increasing hydrocyanic acid. On the contrary, the potential amount may be lessened. Determinations were made on samples collected at sundown and also before sunrise. The data obtained were not conclusive in determining the effect of light on the potential amount of hydrocyanic acid present.

## HYDROCYANIC ACID IN SUDAN HAY

Two samples were taken from the outside of a stack of Sudan hay and two from the inside. No hydrocyanic acid was found.

## AMOUNT OF HYDROCYANIC ACID IN OTHER SORGHUMS

On July 23 a sample of kafir was taken and separated into leaves and stems. From the leaves were obtained 16 mgm., and from the stems 10 mgm. of hydrocyanic acid. The kafir stems were very little developed. Sudan grass, tested the same day, gave 8 mgm. of the acid from the same weight of material. On July 26, just after a heavy rain, following a period of dry weather, a sample of kafir gave 72 mgm. and a sample of sorgo (cane) 42 mgm. of hydrocyanic acid. Sudan grass 6 inches high, tested on that date, gave 32 mgm. of hydrocyanic acid.

On August 7 a sample of second-growth sorgo (cane) was received from LaHarpe, Kans. About one-fifth was quite dry, two-fifths were wilted and yellow, and two-fifths were green. The sample was somewhat moldy. One portion digested in the usual manner gave 13 mgm. hydrocyanic acid. Another portion distilled at once from hot water gave 24 mgm., showing that the hydrocyanic acid was in free condition. Another sample of sorgo was sent in from Seneca, Kans. This was reported to have killed six cows. From the portion distilled from hot water 20 mgm. were obtained and from the portion digested in the usual way 36 mgm.

On September 2 a quantity of Red Amber kafir was collected, and six portions were prepared and treated with the results given in Table XVII.

TABLE XVII.—*Hydrocyanic acid in Red Amber kafir*

| Sample No. | Treatment. | HCN |
|---|---|---|
| | | *Mgm.* |
| 1 | Left in flask 15 minutes after maceration, after which hot water was added and distilled | 32 |
| 2 | Digested overnight in water | 119 |
| 3 | Digested overnight in *N/0.5* $H_2SO_4$ | None. |
| 4 | Digested overnight in *N/0.1* $H_2SO_4$ | None. |
| 5 | Digested overnight in *N/0.01* $H_2SO_4$ | 40 |
| 6 | Digested overnight in *N/0.01* NaOH | 36 |

Part of this experiment was repeated by putting macerated sorgo into boiling water at once. This gave 8 mgm. hydrocyanic acid, while that digested overnight gave 96 mgm. Another portion was divided into five portions. After maceration they were all digested in water overnight. Then to these portions standardized sulphuric acid was added so as to make the normalities indicated. The results are given in Table XVIII.

These determinations show without a doubt that sorgo and kafir contain much larger amounts of hydrocyanic acid than does Sudan grass, and also that the conditions for obtaining it are very similar.

TABLE XVIII.—*Effect of different treatments on liberation of hydrocyanic acid in sorgo*

| Sample No. | Treatment. | HCN |
|---|---|---|
| | | *Mgm.* |
| 1 | Digested in water and distilled | 72 |
| 2 | Digested in water and distilled | 80 |
| 3 | Digested in water and distilled from $N/0.5$ $H_2SO_4$ | 64 |
| 4 | Digested in water and distilled from $N/0.1$ $H_2SO_4$ | 72 |
| 5 | Digested in water and distilled from $N/0.02$ $H_2SO_4$ | 89 |

## EFFECT OF HYDROCYANIC ACID FROM GREEN SORGO ON A HORSE

Ten-pound portions of green sorgo, testing the amount of hydrocyanic acid given in Table XVIII, were fed to a horse. No effect on respiration, pulse, or temperature could be observed by Dr. H. F. Lienhardt, of the Veterinary Division, who made the observations. Data presented in this paper show that such a degree of acidity as is found in the stomach of a horse would prevent liberation of hydrocyanic acid from the green material. Feeding wilted sorgo was not tried.

## SUMMARY

(1) In this paper are presented data giving the results of tests made on Sudan grass for hydrocyanic acid during the summer and early fall of 1920.

(2) The maximum amount of hydrocyanic acid was obtained by macerating the material and digesting in water at room temperature for about six hours or overnight.

(3) Practically all the hydrocyanic acid was found in the leaves. In well-developed stems none was found.

(4) More hydrocyanic acid was found in younger plants than in those more mature. This is due mostly to stem development. If leaves only are used the differences are small, except when the plants approach maturity. More was found in the summer than in the fall.

(5) Hydrocyanic acid does not exist as free HCN in the growing plant. It begins to be liberated as soon as the plant is macerated or undergoes wilting.

(6) Liberation of hydrocyanic acid is intimately associated with enzym action. If this enzym action is inhibited by addition of hot water or acids, no hydrocyanic acid will be liberated. Hydrocyanic acid was obtained from wilted grass when hot water was added, because during the wilting process hydrocyanic acid was set free.

(7) Hydrocyanic acid can not be set free from the green material by acids.

(8) The action of strong alkali is similar to that of acids.

(9) Most hydrocyanic acid is present when the plant is in a vigorous growing condition.

(10) Sudan grass contains less hydrocyanic acid than sorgo or kafir.

# NUTRIENT REQUIREMENTS OF GROWING CHICKS: NUTRITIVE DEFICIENCIES OF CORN[1]

By F. E. Mussehl, *Professor of Poultry Husbandry*, J. W. Calvin, *Associate Chemist, Nebraska Agricultural Experiment Station*, with the cooperation of D. L. Halbersleben and R. M. Sandstedt

Investigators in the field of nutrition have noted that chickens behave unlike rats and swine when limited to rations of corn or wheat grains and their products. This fact has made necessary the planning and execution of experimental work having for its object a determination of the values and deficiencies of our common feeding stuffs when used for poultry and egg production. The results of a series of experiments carried on at this Station with this objective are reported in this paper.

From the experience of investigators[2] who have worked with other species, mainly rats and swine, it has seemed that systematic inquiry should be made into the (a) ash requirements, (b) protein requirements (quality and quantity), and (c) food accessory requirements. Earlier investigational work with chicks by Osborne and Mendel[3] and Hart, Halpin, and Steenbock[4] indicates that another element, (d) the physical factor, is also of fundamental importance and must be considered in any complete study of the nutritive values of a particular grain or ration.

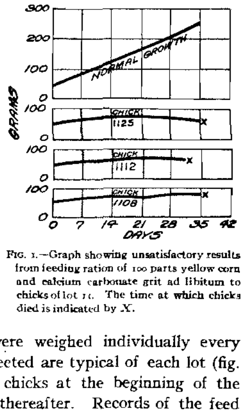


FIG. 1.—Graph showing unsatisfactory results from feeding ration of 100 parts yellow corn and calcium carbonate grit ad libitum to chicks of lot 1. The time at which chicks died is indicated by X.

In our work 10-day-old Single-Comb White Leghorn chicks were used, special care being taken to select for vigor, vitality, and uniformity in each lot. Nine chicks per lot were used for the first series of experiments. Chicks were weighed individually every seven days, and the growth curves selected are typical of each lot (fig. 1-11). They show the weight of the chicks at the beginning of the experiment and the change in weight thereafter. Records of the feed

[1] Published with the approval of the Director of the Nebraska Agricultural Experiment Station.

[2] McCollum, E. V., Simmonds, N., and Pitz, W. THE RELATION OF THE UNIDENTIFIED DIETARY FACTORS, THE FAT-SOLUBLE *a*, AND WATER-SOLUBLE *b*, OF THE DIET TO THE GROWTH-PROMOTING PROPERTIES OF MILK. *In* Jour. Biol. Chem., v. 27, no. 1, p. 33-43, 6 charts (1-3, 6 in text). 1916.

[3] Osborne, Thomas B., and Mendel, Lafayette B. THE GROWTH OF CHICKENS IN CONFINEMENT. *In* Jour. Biol. Chem., v. 33, no. 3, p. 433-438, pl. 4-6. 1918.

[4] Hart, E. B., Halpin, J. G., and Steenbock, H. USE OF SYNTHETIC DIETS IN THE GROWTH OF BABY CHICKS. A STUDY OF LEG WEAKNESS IN CHICKENS. *In* Jour. Biol. Chem., v. 43, no. 2, p. 421-442, 2 pl. 1920.

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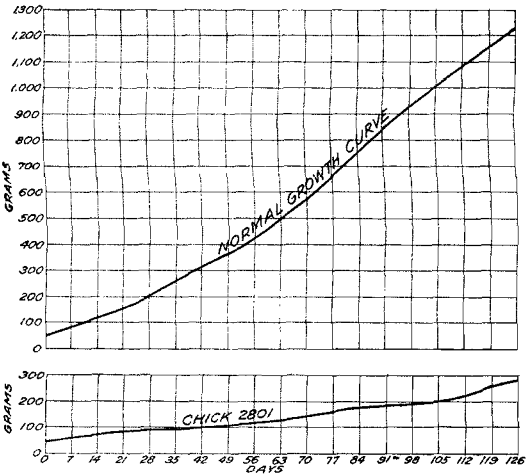


FIG. 2.—Graph showing slow but continuous growth of chick in lot 211, fed ration of 95 parts yellow corn and 5 parts ash mixture

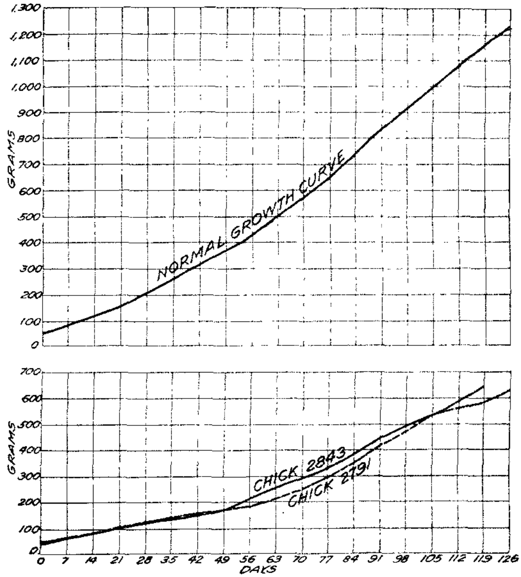


**FIG. 3.—Graph showing** growth of chicks in lot 213, fed ration of 80 parts yellow corn, 15 **parts casein,** and 5 parts ash mixture.

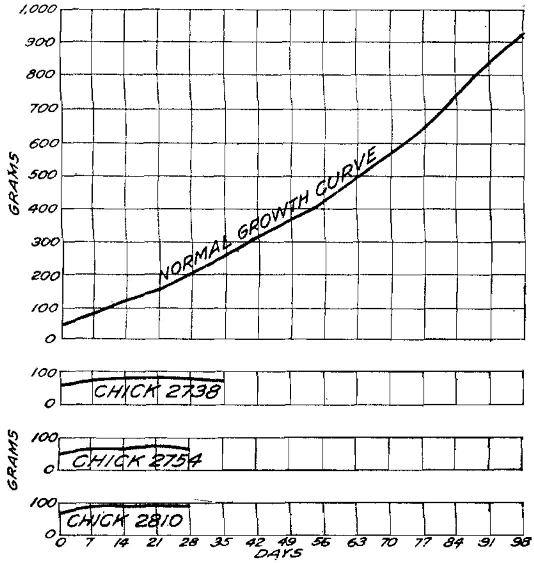


FIG. 4.—Graph showing detrimental results of adding 5 parts butter fat to ration of 65 parts yellow corn, 15 parts casein, 5 parts ash mixture, and 10 parts starch for chicks in lot 215.

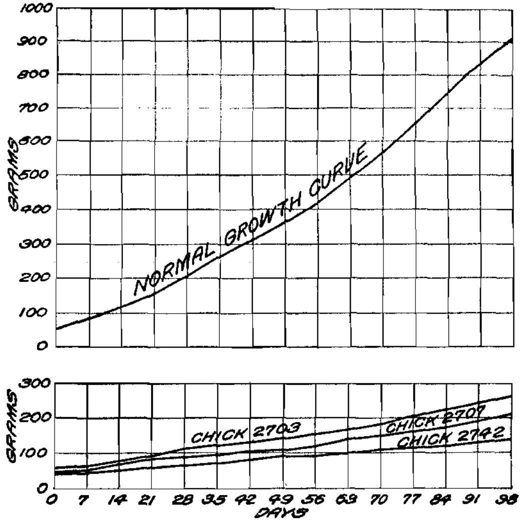


FIG. 5.—Graph showing that the addition of 20 parts corn gluten did not improve ration of 65 parts yellow corn, 5 parts ash mixture, and 10 parts starch for chicks of lot 708.

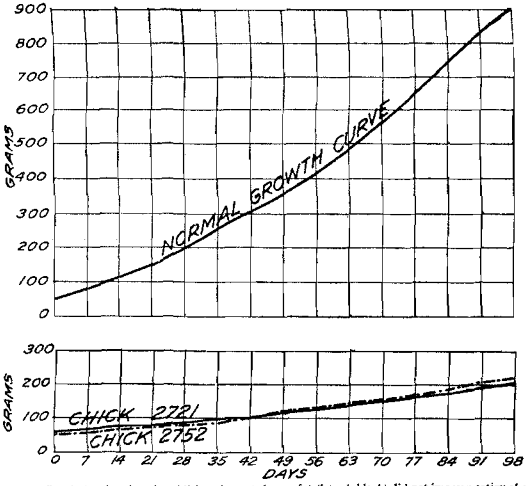


FIG. 6.—Graph showing that the addition of 5 parts butter fat (fat-soluble A) did not improve ration of 5 parts yellow corn, 20 parts corn gluten, 5 parts ash mixture, and 5 parts starch for chicks of lot 209.

consumption of each lot were also obtained. Clean wood shavings were used for litter, and each lot was confined to a pen 2 by 8 feet in size until the chicks were 8 weeks old, when the near normal lots were given a yard 4 by 8 feet in size.

Methods of feeding are known to have considerable influence on the efficiency of a ration; so a standard policy was established of dividing the ration into two parts—the scratch or coarse feed and the mash or fine feed. The rations were mixed so that equal quantities of mash and scratch feeds were provided. The mash feed contained all the supplemental ingredients, such as ash, butter fat, and purified casein.

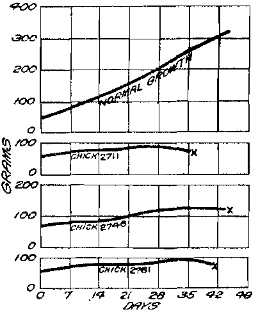


FIG. 7.—Graph showing that the addition of 15 parts soybean meal did not improve ration of 80 parts yellow corn and 5 parts ash mixture for chicks of lot 216 The time at which chicks died is indicated by X

The casein used in the rations was purified by extracting repeatedly with distilled water slightly acidified with acetic acid. After extraction the casein was drained and dried in an air oven at 70° to 100° C. and then ground. The butter fat was purified by melting at 40° in a water bath and was then centrifuged to remove ash, casein, and other material. The ash mixture[1] used in our rations was composed of the following ingredients, parts by weight:

| | |
|---|---|
| Bone ash | 50 |
| Calcium carbonate | 14 |
| Sodium chlorid | 15 |
| Dipotassium phosphate | 10 |
| Calcium lactate | 5 |
| Magnesium sulphate | 3 |
| Sulphur | 2 |
| Iron sulphate | 1 |

The results of our inquiries may briefly be summarized as follows:

(1) Yellow corn (maize) is deficient in several of the essential qualities necessary for the complete nutrition of growing chicks. A deficiency in the ash content of the yellow corn kernel is no doubt responsible for the early failure of baby chicks when restricted to a ration of corn alone. Supplementing the corn kernel with 5 per cent of a complete ash mixture improved the ration so as to enable very slow but persistent growth.

[1] PHILIPS, A. G., CARR, R. H., and KENNARD, D. C. MEAT SCRAPS VERSUS SOY-BEAN PROTEINS AS A SUPPLEMENT TO CORN FOR GROWING CHICKS. *In* Jour Agr. Research, v. 18, no. 7, p. 391–398, 1 fig., pl. 50. 1920

(2) Yellow corn is deficient in quality and quantity of protein required for normal growth of chicks. The addition of more corn protein by including corn gluten in the ration did not markedly improve the efficiency of the ration. Compare growth curves, lots 208 and 211.

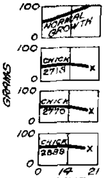


FIG. 8.—Graph showing bad results from lack of roughage in ration of 65 parts yellow corn, 15 parts gelatin, 5 parts butter fat, 5 parts ash mixture, and 10 parts starch for chicks of lot 217. The time at which chick died is indicated by X.

(3) The addition of 15 per cent purified casein to a basal ration of yellow corn and ash did improve the ration decidedly. Compare growth curves, lots 211 and 213. The amino acid deficiencies of the corn proteins are no doubt supplemented by the amino acid contributions of the casein.

(4) Supplementing the basal yellow corn ration with certain other proteins, egg albumen, and gelatin, lowered rather than raised the efficiency of the ration. The poor results with rations 217 and 218 were probably due to a distinctly sticky physical quality which prevented normal nutrition.

(5) The fat-soluble food accessory does not appear to be a limiting factor in a yellow corn diet for baby chicks. The addition of butter fat to a yellow corn, casein, and ash ration (lot 215) did not improve but rather lowered the efficiency of the ration. A slight change in the physical condition of the ration may explain this lowered efficiency, though it is more probable that the butter fat addition temporarily stimulated growth so that the supply of some other essential accessory was exhausted earlier than would have been the case had the butter fat been omitted. It is apparent at least that the failure of chicks on ration 215 was not due to fat-soluble A starvation.

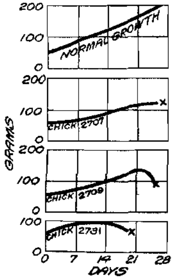


FIG. 9.—Graph showing that because proper physical quality was lacking the addition of 15 parts egg albumen did not improve ration of 65 parts yellow corn, 5 parts butter fat, 5 parts ash mixture, and 10 parts starch for chicks of lot 218. The time at which chicks died is indicated by X.

(6) Green feeds make certain very valuable contributions to a ration for growing chicks. The addition of wheat greens to a yellow corn, casein, and ash ration effected a decided improvement in the efficiency of the ration. An excess of the wheat greens was offered, and subsequent observations indicate that about 5 per cent (dry matter basis) of this kind of green food are consumed when offered regularly in excess. The helpful influence of the wheat greens may have been due to, first, an improvement of the physical condition of the ration; second, a food accessory con-

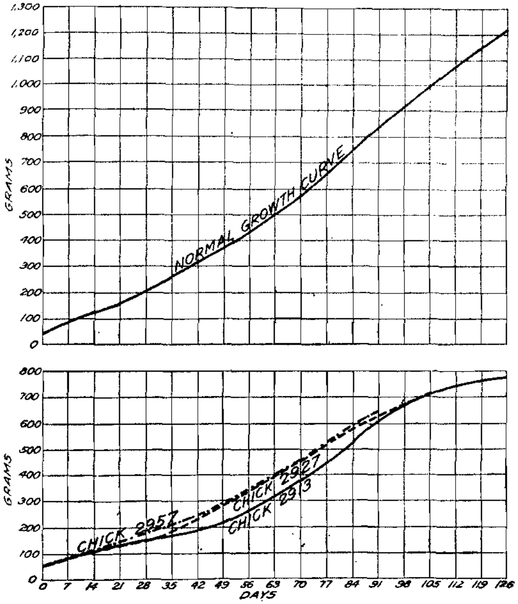


FIG. 10.—Graph showing that the addition of excess of wheat greens improved ration of 80 parts yellow corn, 15 parts casein, and 5 parts ash mixture for chicks of lot 227.

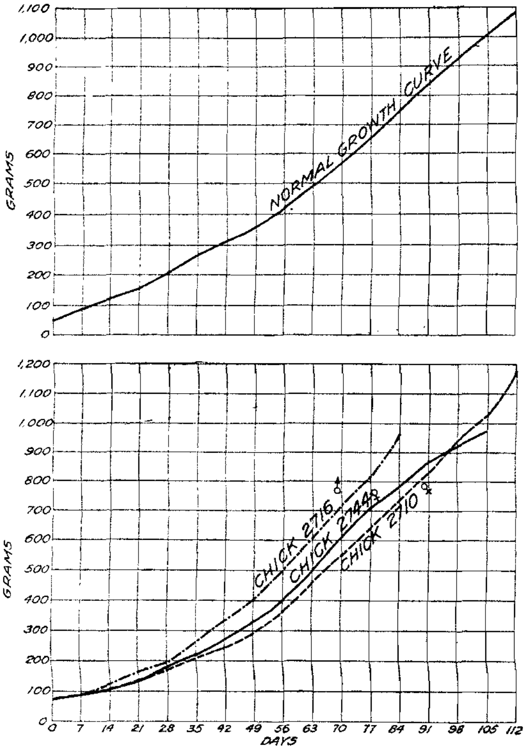


FIG. 11.—Graph showing normal growth produced by ration of 65 parts wheat, 15 parts casein, 5 parts butter fat, 5 parts ash mixture, 10 parts starch, and excess of wheat greens for chicks of lot 205. Birds were kept on this ration until they produced eggs, at age of about 200 days.

tribution; third, an increased food consumption, due to a stimulatory effect on the appetite. A series of experiments to shed further light on the specific contributions which green feeds make to the ration is in progress at this time.

(7) It is possible to raise to normal maturity chicks confined to a small pen. Drummond [1] reports great difficulty in rearing chicks in confinement, and other investigators have noted some of the problems, especially leg weakness.[2] Our lot 205 grew to normal maturity, some of the pullets producing eggs when about 200 days old, though never having more range than was provided in a yard 4 by 8 feet in size. Ration 205, though not synthetic, is of interest because of its comparative simplicity.

[1] DRUMMOND, Jack Cecil. OBSERVATIONS UPON THE GROWTH OF YOUNG CHICKENS UNDER LABORATORY CONDITIONS. *In* Biochem. Jour., v. 10, no. 1, p. 77-88, 1 pl. 1916

[2] HART, E. B., HALPIN, J. G., and STEENBOCK, H. OP. CIT.

# AECIAL STAGE OF THE ORANGE LEAFRUST OF WHEAT, PUCCINIA TRITICINA ERIKS.[1]

By H. S. Jackson, *Chief in Botany*, and E. B. Mains, *Associate Botanist, Purdue University Agricultural Experiment Station, and Agents, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*[2]

This paper presents, in part, the results of a study of the leafrusts of wheat, rye, barley, corn, and related grasses which was begun in 1918. One of the important phases of this investigation is the determination of the aecial relationships of the various races or species included in the collective species, *Puccinia Clematidis* (DC.) Lagerh. (*P. Agropyri* Ellis and Ev.), and other closely related forms. While a number of the rusts of this group which occur on wild grasses have been connected with aecia, their host limitations and interrelations are not well understood. This study is especially important in the case of the leafrust of wheat, *P. triticina* Eriks. So long as the aecial stage of this species was unknown, little progress could be made in developing our knowledge with reference to its origin, development, spread, and relation to other rusts. The results of the investigation of the aecial relationship of this rust are presented in the following pages.

## HISTORICAL REVIEW

Three rusts are known to attack wheat: the black or stemrust, *Puccinia graminis* Pers.; the stripe or yellow rust, *P. glumarum* (Schmidt) Eriks. and Henn.; and the orange or leafrust, *P. triticina*. Of these the stemrust is the only one for which the aecial stage has been determined. This rust was shown by De Bary to have its aecial stage on *Berberis vulgaris* L., and this relationship has since been demonstrated repeatedly by a number of workers in various parts of the world. The discovery of the place of *Aecidium Berberidis* Pers. in the life cycle of *P. graminis* caused De Bary (*4, p. 207–211*)[3] to turn his attention to the study of other grass rusts having incomplete life cycles. This resulted in the discovery that *P. rubigo-vera* (DC.) Wint. (*P. straminis* Fckl.) on rye was connected with aecia on *Anchusa officinalis* and *Anchusa arvensis*. Sowings made with teliospores from rye resulted in the production of

[1] Published with the approval of the Director as a contribution from the Department of Botany, Purdue University Agricultural Experiment Station. Cooperative investigation between the Purdue University Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

[2] The writers wish to acknowledge their indebtedness to various pathologists throughout the country for aid in obtaining material for the cultural studies upon which this paper is based, and to Mr. Forest Fuller, temporary culture assistant during the spring of 1919, and Mr. Emile Mardfin and Miss Florence M. Smith, Scientific Assistants, Office of Cereal Investigations, Bureau of Plant Industry, for assistance in carrying out the cultural investigations.

[3] Reference is made by number (italic) to "Literature cited," p. 170–171.

aecia on Anchusa, and when sowings were made with aeciospores from Anchusa, uredinia on rye were developed. Sowings of basidiospores from rye upon *Berberis vulgaris* Hol., *Rhamnus Frangula*, *Rhamus cathartica*, *Ranunculus acris*, *Ranunculus bulbosus*, *Taraxacum officinalis*, and *Urtica dioica* were without result. Nielsen (*20, p. 37*) 10 years later reported obtaining infection with aeciospores from *Anchusa officinalis* on both rye and wheat. Plowright (*21, p. 168*) states that in the fall of 1885 he obtained aecia upon *Anchusa arvensis* by placing wheat straw rusted with *P. rubigo-vera* near that host.

At the time this work was carried out the name *Puccinia rubigo-vera* was used for the leafrusts of wheat, rye, and barley, as well as for similar grass rusts having globoid urediniospores and long covered telia. Eriksson and Henning (*11, p. 197–203, 257–250*) separated this species into two—*Puccinia glumarum*, the stripe rust, and *Puccinia dispersa* Eriks., the brown rust. Under the latter they included the rust of wheat as well as that of rye. The rust of wheat, however, was considered as a forma specialis, *Tritici*, of *Puccinia dispersa*. As the leafrust of rye had been shown by De Bary (*4*) to be connected with aecia on Anchusa, Eriksson (*10, p. 254–257*) sought for the same connection for the leafrust of wheat. His sowings of basidiospores from wheat upon *Anchusa officinalis* and *A. arvensis*, however, produced no infection, as was also the case when aeciospores from Anchusa were sown on wheat. No results were obtained when basidiospores were sown on *Nonnea rosea*, *Myosotis arvensis*, *M. alpestris*, *Symphytum asperrimum* and *Pulmonaria officinalis*, species of Boraginaceae related to Anchusa upon which unconnected aecia were known to occur. As a result of these cultures, Eriksson (*10, p. 270*) concluded that the orange leafrust of wheat was a distinct species and gave it the name, *Puccinia triticina*.

Klebahn (*17, p. 85–86; 18, p. 246*) made rather extensive cultures in an endeavor to discover the aecial host of *Puccinia triticina*. Besides sowing aeciospores of *Aecidium Anchusae* Eriks. and Henn. on wheat he made sowings of basidiospores on *Anchusa arvensis* and *Anchusa officinalis* without result. Sowings of basidiospores also were made without success upon *Triticum vulgare*, *Ranunculus acer*, *Ranunculus asiaticus*, *Ranunculus auricomus*, *Ranunculus bulbosus*, *Ranunculus Ficaria*, *Ranunculus flammula*, *Ranunculus lanuginosus*, *Ranunculus repens*, *Anemone ranunculoides*, *Aconitum Lycoctonum*, *Aconitum Napellus*, *Berberis vulgaris*, *Nasturtium* sp., *Barbaraea vulgaris*, *Melandryum album*, *Coronaria flos-cuculi*, *Agrostemma Githago*, *Rhamnus cathartica*, *Lythrum Salicaria*, *Ribes Grossularia*, *Aegopodium Podagraria*, *Pastinaca sativa*, *Valeriana dioica*, *Knautia arvensis*, *Tussilago Farfara*, *Taraxacum officinale*, *Centaurea Cyanus*, *Achillea Ptarmica*, *Campanula rotundifolia*, *Ligustrum vulgare*, *Phillyrea* sp., *Echium vulgare*, *Lithospermum purpureo-coeruleum*, *Myosotis* sp., *Symphytum officinale*, *Glechoma hederacea*, *Prunella vulgaris*,

*Rumex acetosa*, and *Urtica dioica*. These results substantiate those obtained by Eriksson and indicate that the positive results reported by Nielsen (*20*) and Plowright (*21*) were probably due to a mixture of rusts or of hosts.

The failure to obtain infection on Boraginaceous hosts has influenced other workers to turn their attention to other families in a search for the aecial hosts. Arthur (*1, v. 9, p. 304*), largely as a result of morphological studies, reached the conclusion that *Puccinia triticina* was best considered a race of *P. Agropyri*, and upon this basis Arthur and Fromme (*3, p. 333–337*) have placed it in the collective species *Dicaeoma Clematidis* (DC.) Arth. Several races of this collective species had been shown by workers in Europe and America to go to species of Clematis. Arthur thought that the aecial host might be either *Clematis flammula* or *C. vitalba* as these were the only common species of Clematis found in the wheat-growing regions of southern Europe, northern Africa, and western Asia, a region which at that time was considered as the probable home of the original wild wheat. His culture with wintered telia of the leaf-rust of wheat on *C. flammula*, however, was unsuccessful.

According to Butler (*6, p. 15*) Cunningham and Prain (*9*) considered that there was considerable ground for believing that an Aecidium on *Launaea asplenifolia*, one of the Cichoriaceae, was the aecial stage of *Puccinia triticina*, as it was found throughout the greater part of the wheat-growing area of India. Butler, however, sowed aeciospores from this host upon wheat without obtaining infection.

These unsuccessful attempts to demonstrate an aecial stage for *Puccinia triticina* have resulted in the development of the idea that the aecial stage of this rust has been lost and that it is able to maintain itself without one. In this connection a number of important facts have been established and a number of interesting hypotheses proposed. It has been shown by Bolley (*5, p. 13–14*), Hitchcock and Carleton (*15, p. 1–2*), Carleton (*8, p. 21–22*), and others that in certain regions, *P. triticina* is able to overwinter by means of its uredinal mycelium and that no aecial host is necessary for the maintenance of this species. This does not appear, however, to be true for all regions where *P. triticina* is abundant (*6, p. 11*). A number of suggestions have been made to explain the yearly appearance of the rust in regions where the urediniospores or uredinial mycelium does not overwinter. It was considered possible that spores may be carried from other regions by the wind. The mycoplasm theory of seed transmissal has also been put forward as a possible explanation. Whatever may be the merits of these hypotheses, they have resulted in recent years in directing attention away from a search for the aecial host of this species.

## BASIS OF CULTURAL INVESTIGATIONS

A study of *Puccinia triticina* in comparison with other grass rusts with long covered telia shows that it can not be readily separated morphologically from the leafrust of rye. The separation of this form as a species was made by Eriksson (*10*) because he obtained only slight infection on rye with urediniospores and was not able to obtain infection on Anchusa with basidiospores and because the teliospores germinated in the spring, while those of the rye rust germinated in the fall. The close morphological similarity, however, furnished considerable grounds for the assumption that the aecial host of leafrust of wheat was likely to be some species of Boraginaceae other than Anchusa, especially as another rust of this type, *Puccinia bromina* Eriks., has since been found to have its aecia on the Boraginaceous hosts *Symphytum officinale* and *Pulmonaria montana*, with very weak development of aecia on Anchusa (*19, p. 182–202*). Unfinished investigations now being conducted in this laboratory strongly indicate that in America certain grass rusts having aecia on Boraginaceous hosts are very similar to the leafrust of wheat and rye. For these reasons it was considered desirable to test as many Boraginaceous hosts as were available, as possible aecial hosts for the leafrust of wheat.

There is, however, still another group of grass rusts very similar to the orange leafrust of wheat to which Arthur (*1, v. 9, p. 304*) has called attention. This group has aecia upon various Ranunculaceous hosts and includes forms which have been separated from time to time, according to their aecial connection, together with slight morphological variation, into a number of species, including *Puccinia persistens* Plowr., *P. perplexans* Plowr., *P. Agropyri*, and *P. alternans* Arth. The writers felt from the beginning that the greatest possibility of success in the search for the aecial stage was to study thoroughly the genera of this family on which aecia were known to occur.

The idea that *Puccinia triticina* has lost its ability to develop an aecial stage through long propagation by urediniospores, while admittedly possible, was not considered to be fully substantiated.

## CULTURES MADE IN 1919

With these considerations in mind rather extensive sowings were made in the spring of 1919 upon a considerable number of species of the families Ranunculaceae and Boraginaceae and the closely related family Hydrophyllaceae. For this purpose, 20 collections of telia of *Puccinia triticina* were obtained from various sections of the country during the summer and fall of 1918 and placed outdoors to winter. Early in March these began to germinate. Ten of the 20 collections gave good germination and were sown upon various species of the above-named families and upon *Ornithogalum umbellatum* L., *Impatiens* sp., and *Camassia esculenta* (Ker.) Robins. (*Quamasia hyacinthina*). The results obtained are given in Table I.

TABLE I.—*Data obtained in 1919 from sowing teliospores of Puccinia triticina, from 10 different localities, on various host plants, mostly of the families Ranunculaceae and Boraginaceae* [a]

| Host inoculated. | No. 118 (Okla.). | No. 218 (Ala.). | No. 418 (Tenn.). | No. 618 (Ga.). | No. 718 (Ga.). | No. 818 (S. C.). | No. 918 (Ind.). | No. 3518 (Wis.). | No. 3818 (Wis.). | No. 4518 (Wis.). |
|---|---|---|---|---|---|---|---|---|---|---|
| *Aconitum Fischeri* Reich. | | | — | | | | | | | |
| *Aconitum Napellus* L. | | — | | | | | | | | — |
| *Actaea spicata* L. | — | | | | — | | | | | |
| *Anemone canadensis* L. | — | | — | | | | | | — | — |
| *Anemone cylindrica* Gray | — | | | — | | — | — | — | | |
| *Anemone japonica* Sieb. & Zucc | | — | | | — | | — | | | |
| *Anemone* sp. | — | | | | | | | | | |
| *Aquilegia alpina* L. | | — | — | | | | | | | — |
| *Aquilegia canadensis* L. | — | | | — | | — | | | | — |
| *Aquilegia chrysantha* Gray | | | — | | — | | | | — | — |
| *Aquilegia glandulosa* Fisch | — | | | | | — | | | | |
| *Aquilegia Skinneri* Hook | — | | | | | | | | | |
| *Aquilegia vulgaris* L. | — | | — | | — | | | | — | |
| *Aquilegia* sp. | | | — | | | | | | | |
| *Cimicifuga racemosa* (L.) Nutt | — | | | | | — | | | | |
| *Clematis Douglasii* Hook | — | | | | | — | | | | |
| *Clematis Fremontii* Wats | | | | | | | | — | | |
| *Clematis heracleaefolia* DC. | | | | | | | | | | |
| *Clematis ligusticifolia* Nutt. | | | — | | | | | | | |
| *Clematis orientalis* L. | | | — | | | | | | | |
| *Clematis recta* L. | — | — | — | | — | | | | | |
| *Clematis virginiana* L. | — | | — | | — | | | — | | |
| *Clematis* sp. | | | — | | — | | — | | — | |
| *Delphinium ajacis* L. | | | | | — | | | | | |
| *Delphinium* "*Belladonna*" (Hort.) | | | | — | — | | | | | |
| *Delphinium consolida* L. | | | | | — | | | | | |
| *Delphinium Geyeri* Greene | | | — | | — | | | | | |
| *Echium vulgare* L. | — | — | | | | | | | | |
| *Hepatica* sp. | — | | | | | | | | | |
| *Hydrophyllum appendiculatum* Michx | — | | | | — | | | | | — |
| *Impatiens* sp. | — | | | | — | — | — | | — | — |
| *Mertensia virginica* (L.) Link | | — | — | — | | | | | | |
| *Myosotis palustris* Lam | | — | — | | | | | | | |
| *Myosotis scorpioides* L. | — | — | | | | | | | | — |
| *Ornithogalum umbellatum* L. | — | | | | | — | — | | | |
| *Phacelia tanacetifolia* Benth. | | | | | — | | | | | |
| *Phacelia Purshii* Buckl | — | | | | — | | — | | | |
| *Camassia esculenta* (Ker.) Robins. | — | | | | | | | | | |
| *Ranunculus aconitifolius* L. | | — | — | | | | | | — | — |
| *Ranunculus acris* L. | | | | — | | | — | | | |
| *Ranunculus repens* L. | — | | | | | | — | | | |
| *Thalictrum angustifolium* L. | | | | | | | [b] [c]— | | | |
| *Thalictrum aquilegifolium* L. | | | — | | — | | [b] [c]— | | | |
| *Thalictrum dioicum* L. | — | | | | | | — | | | — |
| *Thalictrum minus* L. | | | | | | | — | | | |
| *Thalictrum polygamum* Muhl | | | | | | | | — | | |
| *Thalictrum* sp. | | | | | | | | | — | |
| *Trollius europeus* L. | | | — | | | | | | | — |

[a] — No infection.
[c] Pycnia produced.
[b] Two sowings were made. Pycnia were produced from only one sowing, no result being obtained from the other.

The telial collections used in the cultures were all obtained from *Triticum aestivum* (*T. vulgare*) in the following localities:

118, from Stillwater, Okla., collected by J. D. Moore.
218, from Flint, Ala., collected by McClellan.
418, from Tennessee, collected by W. T. Evans.
618, from Carrolton, Ga., collected by R. O. Burns.
718, from Carrolton, Ga., collected by R. O. Burns.

818, from Anderson, S. C., collected by R. O. Burns.

918, from La Fayette, Ind., collected by E. H. Toole.

3518, from Menah, Wis., collected by E. H. Toole.

3818, from Wisconsin, collected by E. H. Toole.

4518, from Superior, Wisconsin, collected by E. H. Toole.

Negative results were obtained on all but two species of the hosts used. The collection from La Fayette, Ind. (No. 918), gave infection upon *Thalictrum angustifolium* and *T. aquilegifolium*, producing, however, only pycnia. It was impossible to carry this study further in 1919, as the above results were not obtained until late in the spring.

The failure of aecia to develop from the two successful infections could be explained on either of two hypotheses. The conditions in the greenhouse may have been unfavorable, or the species of Thalictrum used may have been resistant. In either case, however, these results were interpreted as indicating that the aecial host of the leafrust of wheat was some species of Thalictrum. There was considerable basis for this assumption. All of the culture studies being carried on in this laboratory with the related rusts, occurring on wild grasses, and having aecia on members of the family Ranunculaceae, have indicated that while a given race may develop aecia on several species in one host genus with varying degrees of virulence it will not go to species of more than one genus. The rusts of this group show a very high degree of specialization. The two species of Thalictrum on which infection was obtained were foreign species, while the North American species, *Thalictrum dioicum* and *T. polygamum*, were not infected. On this account it was thought that the susceptible aecial hosts for the leafrust of wheat probably were foreign species of Thalictrum. As the leafrust of wheat presumably is an introduced form, as explained in the following pages, this would be expected, and on that basis the species of Thalictrum should be western Asiatic or eastern European, corresponding to the region in which wheat is believed to have originated.

## CULTURES MADE IN 1920

In preparation for cultural studies for the spring of 1920 an effort was made during the summer and fall of 1919 to obtain as many species of Thalictrum as possible. It was impossible to obtain material from foreign botanical gardens in time to be of use, and the best that could be done was to secure such species of Thalictrum as were carried by nurserymen in this country, together with such native species as could be obtained through collectors in various parts of the United States. As a result 14 species were brought together. An appeal was also made to the plant pathologists in the various agricultural experiment stations throughout the country for aid in securing telial material of the leafrust of wheat. A very gratifying response to this appeal was made, and in this way 80 collections of telia were obtained and placed out to overwinter. Of these, 51 collections germinated in the spring of 1920 and were sown. The number of collections was so great that it was not possible to sow

them on as large a number of species as was done in 1919, and attention was devoted mainly to sowing upon species of Thalictrum. Of the 51 collections used 9 were sown upon as many Thalictrum species as possible in order to determine the relative susceptibility of these species. The results are given in Table II.

TABLE II.—*Data obtained in 1920 from sowing teliospores of Puccinia triticina from nine different localities on various species of Thalictrum*[a]

| Host inoculated. | Laboratory No. | No. 5619 (Ga.). | No. 6019 (N. C.). | No. 7219 (N. C.). | No. 7819 (Tenn.). | No. 8019 (Mich.). | No. 8719 (Miss.). | No. 11619 (Pa.). | No. 12519 (Idaho). | No. 15119 (Nev.) |
|---|---|---|---|---|---|---|---|---|---|---|
| *Thalictrum angustifolium* L. | 6 | [b]1 | o | o | ... | — | o | [b]1 | — | — |
| *Thalictrum aquilegifolium* L. | 5 | o | — | — | ... | ... | —o | o | ... | [b]1 |
| *Thalictrum aquilegifolium* L. | 66 | — | — | — | | — | —(2) | — | — | — |
| *Thalictrum dasycarpum* Fisch. and Lall | 65 | ... | — | — | ... | ... | | o | — | o |
| *Thalictrum Delavayi* Franchet | 56 | ... | o | 1 | o1 | — | 1 | o | 1 | o |
| *Thalictrum dioicum* L. | 16 | 1 | o | | | — | — | o | | ... |
| *Thalictrum flavum* L. | 53 | 1 | o | 1 | — | 1 | 1 | 1 | 1 | 1 |
| *Thalictrum minus* L. | 17 | — | — | o | [b]1 | — | — | [b]1 | | |
| *Thalictrum minus adiantifolium* | 63 | [b]1 | —(2) | —(2) | o | — | —(3) | — | o | — |
| *Thalictrum occidentale* Gray | 115 | — | — | — | — | ... | ... | — | | ... |
| *Thalictrum polycarpum* S. Wats | 114 | | ... | ... | | | ... | [b]1 | [b]1 | ...... |
| *Thalictrum polygamum* Muhl. | 19 | — | —(2) | — | o | — | —(2) | — | | — |
| *Thalictrum* sp. | 98 | o | — | o1 | ... | o | — | — | o | o |
| *Thalictrum* sp. | 35 | ... | 1 | 1 | 1 | — | ... | 1 | 1 | — |

[a] — No infection.
o Pycnia only produced.
1 Aecia following pycnia.
A numeral in parenthesis following the sign indicates the number of times the results were obtained.
[b] Although aecia were produced, the infection was weak.

The following is a list of the sources of the telial material used in the cultures:

5619, from Athens, Ga., collected by C. A. Ludwig.
6019, from W. Raleigh, N. C., collected by Ludwig and Wolf.
7219, from Hickory, N. C., collected by C. A. Ludwig.
7819, from Tennessee, collected by C. A. Ludwig.
8019, from Coldwater, Mich., collected by B. W. Mains.
8719, from Canton, Miss., collected by C. A. Ludwig.
11619, from State College, Pa., collected by J. T. Adams.
12519, from Moscow, Idaho, collected by C. W. Hungerford.
15119, from Reno, Nev., collected by G. R. Hoerner.

In addition to the sowings indicated in Table II, culture 8719 was sown upon *Aquilegia glandulosa* Fisch., *A. olympica* Boiss., *Clematis heraclaefolia* DC., *C. paniculata* Thunb., *C. recta* L., and *Ranunculus acris* L., all without infection.

An examination of Table II shows that 12 out of the 14 species of Thalictrum were infected, *Thalictrum occidentale* and *T. aquilegifolium* apparantly being immune. The species on which infection occurred showed varying degrees of susceptibility. *Thalictrum dasycarpum* and

*olygamum* gave mostly negative results or the occasional production ia. *T. angustifolium*, *T. aquilegifolium* (5), *T. minus*, *T. minus olium*, and *T. polycarpum* showed occasionally a weak development of aecia, but usually only pycnia developed or no infection occurred. *T. dioicum*, in one case, showed a moderate development of aecia; in all other cases only pycnia developed, or no infection resulted. *T. Delavayi* and *T.* sp. (98) (Pl. 21, A, B) showed fairly vigorous infection, accompanied in most cases by more or less hypertrophy and usually by well-developed aecia. *T. flavum* (Pl. 21, C) and *T.* sp. (55) (Pl. 21, D) showed a very vigorous infection accompanied usually by pronounced hypertrophy of the infected leaf and petiole tissue and practically always with the production of well-developed aecia.

An attempt has been made to check the determination of the species of Thalictrum used in these studies, but this has been difficult because a number of them have produced neither flowers nor fruit, and the leaf characters in this genus are in most cases extremely variable. Specimens of most of the species have been sent to Mr. S. F. Blake, of the Bureau of Plant Industry, Washington, D. C., who has kindly compared them with specimens in the United States National Herbarium and has given his opinion as to the identity of our material. The following list gives the species used above, their sources, and native distribution as accurately as they could be determined. The accession number of this laboratory follows the name of each species.

*Thalictrum angustifolium* L. (6). Source: Seed from Brooklyn Botanic Garden. Distribution: Central Europe and Asia Minor.

*Thalictrum aquilegifolium* L. (5 and 66). Source: Bobbink and Atkins Nursery Co. Distribution: Europe, Middle and Northern Asia. (No. 66 was purchased for *T. paniculatum*.)

*Thalictrum dasycarpum* Fisch. and Lall. (65). Source: Department of Botany, Michigan Agricultural College. Distribution: Northern and central United States and southern Canada.

*Thalictrum Delavayi* Franchet (56). Source: Farr Nursery Co. Distribution: Western China.

*Thalictrum dioicum* L. (16). Source: LaFayette, Ind. Distribution: Eastern United States.

*Thalictrum flavum* L. (53). Source: Farr Nursery Co. Distribution: Europe, Western Asia, and Asia Minor.

*Thalictrum minus* L. (17). Source: An American nursery. Distribution: Europe, Asia, and eastern and southern Africa.

*Thalictrum minus adiantifolium* (63). Source: Seed from Brooklyn Botanic Garden. Distribution: See *T. minus*.

*Thalictrum occidentale* Gray (115). Source: Corvallis, Oreg. Distribution: Mountains, California to British Columbia.

*Thalictrum polycarpum* S. Wats. (114). Source: Berkeley, Calif. Distribution: California.

*Thalictrum polygamum* Muhl. 19. Source: Ithaca, N. Y. Distribution: Eastern United States.

*Thalictrum* sp. (98). Source: Palisade Nursery Co. Distribution: Exotic.

*Thalictrum* sp. (55). Source: Farr Nursery Co. Distribution: Exotic.

From the data presented above it is evident that a number of species of Thalictrum are susceptible hosts for *Puccinia triticina*. As far as the host determinations are at all certain, the evidence would indicate that the most susceptible hosts are from western Asia and eastern Europe, and doubtless in this region other species will be found of as great or greater susceptibility.

The remaining collections of telia showing good germination were sown on one or more of the susceptible species of Thalictrum in order to determine how uniformly *Puccinia triticina* from the United States would go to Thalictrum. Table III gives the results of these cultures.

TABLE III.—*Data obtained in 1920 from sowing teliospores of Puccinia triticina, from many different localities, on four especially susceptible species of Thalictrum* [a]

| Number and source of telia. | *T.* sp. (55). | *T. Delavayi* (56). | *T. flavum* (54). | *T.* sp. (98) |
|---|---|---|---|---|
| 5119 Pa. | ...... | 1 | ...... | ...... |
| 11719 Pa. | ...... | ...... | ...... | o |
| 0819 W. Va. | ...... | ...... | ...... | 1— |
| 6319 N. C. | ...... | 1 | ...... | o |
| 6419 N. C. | ...... | o | ...... | ...... |
| 7319 N. C. | ...... | o | ...... | ...... |
| 5819 S. C. | ...... | ...... | ...... | 1— |
| 5019 Ga. | ...... | ...... | ...... | 1—(2) |
| 4119 Ala. | ...... | ...... | ...... | o |
| 4519 Ala. | ...... | ...... | 1 | ...... |
| 4419 Tenn.[b] | ...... | ...... | ...... | — |
| 17419 Tenn. | ...... | — | ...... | 1 |
| 6519 Ky. | ...... | o | ...... | ...... |
| 4819 Ind. | ...... | 1 | ...... | ...... |
| 5319 Ind. | ...... | ...... | ...... | 1 |
| 10119 Ind. | ...... | 1 | ...... | ...... |
| 10419 Ill. | ...... | ...... | ...... | 1 |
| 12419 Minn. | ...... | ...... | ...... | 10 |
| 3619 Iowa | ...... | ...... | ...... | o |
| 9219 Mo. | ...... | ...... | ...... | o |
| 9319 Mo. | ...... | o | ...... | ...... |
| 9519 Mo. | ...... | ...... | ...... | o |
| 9619 Mo. | 1 | ...... | ...... | ...... |
| 3819 La. | ...... | ...... | ...... | o |
| 6719 Tex. | ...... | ...... | ...... | 1 |
| 7019 Tex.[c] | ...... | ...... | ...... | o |
| 3219 Ariz. | ...... | ...... | ...... | 1 |
| 6119 Calif. | ...... | o | ...... | ...... |
| 12219 Wash. | ...... | ...... | ...... | o |
| 16519 Wash. | ...... | 1 | ...... | 1 |
| 19919 Wash. | 1 | ...... | ...... | ...... |
| 13919 Oreg. | ...... | ...... | ...... | o |

[a] — No infection.
o Pycnia produced.
1 Aecia following pycnia.
A numeral (in parenthesis) following the sign indicates the number of times the results were obtained.
[b] Sown also on *T. angustifolium*, producing pycnia, and on *T. aquilegifolium* without results.
[c] Sown also on *T. dasycarpum* without results.

Source of telial material used in cultures:

5119, from York, Pa., collected by F. D. Kern.
11719, from Bradford County, Pa., collected by E. T. Nixon.
9819, from Morgantown, W. Va., collected by N. J. Giddings.
6319, from Statesville, N. C., collected by C. A. Ludwig.
6419, from Statesville, N. C., collected by C. A. Ludwig.
7319, from Biltmore, N. C., collected by C. A. Ludwig.
5819, from Clemson College, S. C., collected by C. A. Ludwig.
5019, from Tifton, Ga., collected by C. A. Ludwig.
4119, from Bay Minette, Ala., collected by C. A. Ludwig.
4519, from Auburn, Ala., collected by C. A. Ludwig.
4419, from Union City, Tenn., collected by Carl Kurtzweil.
17419, from Johnson City, Tenn., collected by C. A. Ludwig.
6519, from Lexington, Ky., collected by R. S. Kirby.
4819, from Mount Vernon, Ind., collected by E. B. Mains.
5319, from Washington County, Ind., collected by H. S. Jackson.
10119, from La Fayette, Ind., collected by E. B. Mains.
10419, from Bloomington, Ill., collected by Koehler and Toole.
12419, from Wasioja, Minn., collected by G. W. Martin.
3619, from Ames, Iowa, collected by I. E. Melhus.
9319, from Columbia, Mo., collected by W. E. Maneval.
9419, from Columbia, Mo., collected by W. E. Maneval.
9519, from Columbia, Mo., collected by W. E. Maneval.
9619, from Columbia, Mo., collected by W. E. Maneval.
3819, from Baton Rouge, La., collected by Thiel and Ludwig.
6719, from Dallas, Tex., collected by W. H. Ballamy.
7019, from San Antonio, Tex., collected by R. S. Kirby.
3219, from Yuma, Ariz., collected by L. Y. Leonard.
6119, from Chico, Calif., collected by R. M. Kelia.
12219, from Dayton, Wash., collected by J. W. Hotson.
16519, from Colton, Wash., collected by J. W. Hotson.
19919, from Puyallup, Wash., collected by G. R. Hoerner.
13919, from Oregon, collected by G. R. Hoerner.

The data in this table, taken with those in Table II, show that *Puccinia triticina* from Pennsylvania, West Virginia, North Carolina, South Carolina, Georgia, Alabama, Mississippi, Tennessee, Kentucky, Indiana, Michigan, Illinois, Minnesota, Iowa, Missouri, Louisiana, Texas, Arizona, California, Washington, Oregon, Idaho, and Nevada gave positive results when sown upon Thalictrum.

The following collections showed some germination but produced no infection when sown on Thalictrum:

11819, from Hopkinsville, Ky., collected by Carl Kurtzweil.
9419, from Columbia, Mo., collected by W. E. Maneval.
3919, from Fayetteville, Ark., collected by H. R. Rosen.
5419, from Memphis, Tenn., collected by A. F. Thiel.

7519, from Southampton, N. Y., collected by H. S. Jackson.
8319, from Rocky Ford, Colo., collected by J. G. Leach.
10019, from Buffalo, Minn., collected by G. W. Martin.
10519, from Plainview, Nebr., collected by H. W. Thurston.
11019, from Vermillion, Minn., collected by G. W. Martin.
11219, from Newark, Del., collected by T. F. Manns.

Besides the above, the following collections were wintered, but no erminating teliospores were found, and in consequence they were not own.

1419, from Santa Rosa, Calif., collected by H. S. Jackson.
3319, from Sonora, Mexico, near Yuma, Ariz., collected by L. Y. Leonard.
3419, from St. Louis, Mo., collected by E. B. Mains.
3719, from Jackson, Tenn., collected by Kurtzweil and Thiel.
4019, from Corvallis, Oreg., collected by G. R. Hoerner.
5519, from St. Paul, Minn., collected by A. F. Thiel.
6619, from Hiawatha, Kans., collected by W. H. Ballamy.
6819, from Marshall, Mo., collected by R. S. Kirby.
6919, from Guthrie, Okla., collected by R. S. Kirby.
7119, from Wellington, Mich., collected by G. H. Coons.
7919, from Nashville, Tenn., collected by C. A. Ludwig.
9119, from Madison, Wis., collected by E. B. Mains.
10819, from Manhattan, Kans., collected by L. E. Melchers.
11119, from Toledo, Iowa, collected by I. E. Melhus.
11919, from Fort Collins, Colo., collected by J. G. Leach.
12019, from East Lansing, Mich., collected by Acelia M. Leach.
12119, from East Lansing, Mich., collected by Acelia M. Leach.
12319, from Pullman, Wash., collected by F. D. Heald.
13819, from Fort Collins, Colo., collected by J. G. Leach.
21019, from Moscow, Idaho, collected by G. R. Hoerner.
25519, from Murfreesboro, Tenn., collected by Carl Kurtzweil.
25719, from Clarksville, Tenn., collected by Carl Kurtzweil.

The accompanying map (fig. 1) shows the source of collections used . the work together with the results obtained with them at La Fayette, ıd. This map shows that material from the States of Pennsylvania, 'est Virginia, Indiana, Illinois, North Carolina, South Carolina, Georgia, labama, Mississippi, Louisiana, and Texas gave germination uniformly ıd infected Thalictrum in all cases. A region represented by the States Colorado, Oklahoma, Arkansas, Kansas, Nebraska, Minnesota, Iowa, ıd Missouri, with one arm running through Wisconsin into Michigan ıd another through Tennessee into Kentucky, gave material which ually did not germinate or, if germination was obtained, produced fection on Thalictrum in only a few cases. Whether this situation dicates the presence of another strain of the leafrust having different aracteristics as regards its viability and power to infect Thalictrum, whether it means that the season or climate was of such a nature that

teliospores of a low vitality were produced, remains for future investigation to decide. Telial material from the Pacific coast, while not viable in a number of cases, produced infection on Thalictrum in all cases where germination was obtained.

The aecia produced from the following telial collections were sown back upon wheat:

4519, from Auburn, Ala.
4819, from Mount Vernon, Ind.
5019, from Tifton, Ga.
5619, from Athens, Ga.
5819, from Clemson College, S. C.
6019, from W. Raleigh, N. C.
6319, from Statesville, N. C.

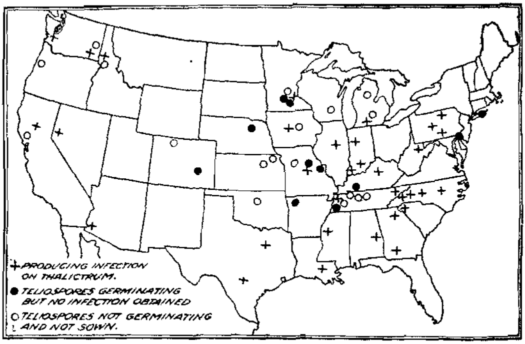


FIG. 1.—Map showing results obtained at LaFayette, Ind., with leafrust material collected in different parts of the United States.

7219, from Hickory, N. C.
7819, from Tennessee.
8019, from Coldwater, Mich.
8719, from Canton, Miss.
9619, from Columbia, Mo.
9819, from Morgantown, W. Va.
10419, from Bloomington, Ill.
11619, from State College, Pa.
12419, from Wasioja, Minn.
12519, from Moscow, Idaho.
15119, from Reno, Nev.
16519, from Colton, Wash.
17419, from Johnson City, Tenn.

These were each sown upon the variety of wheat known as Dawson Golden Chaff, and in all cases positive infection was obtained followed by the development of uredinia which were typical of *Puccinia triticina*.

Sowings of aeciospores also were made upon a number of grasses. Aecia which were produced from telia obtained from Hickory, N. C., Canton, Miss., and Moscow, Idaho, were used and Table IV gives the results.

TABLE IV.—*Results obtained in 1920 from sowing the aeciospores of Puccinia triticina produced from telia obtained in three different areas, on wheat and related grasses*

| Host inoculated. | Number and source of aecia. | | |
|---|---|---|---|
| | No. 7219 (N. C.) | No. 8719 (Miss.) | No. 12519 (Idaho) |
| *Arrhenatherum elatius* (L.) Mert & Koch | | | — |
| *Agropyron caninum* (L.) Beauv | — | — | ........ |
| *Agropyon cristatum* Beauv | ........ | ........ | — |
| *Agropyron desertorum* Schult | ........ | ........ | — |
| *Agropyron inerme* (Schribn. & Sm.) Rydb | ........ | ........ | — |
| *Agropyron repens* (L.) Beauv | — | — | ........ |
| *Agropyron tenerum* Vasey | — | — | ........ |
| *Elymus australis* Schribn. & Ball | — | — | ........ |
| *Elymus canadensis* L | — | — | ........ |
| *Elymus glaucus* Buckl | — | — | ........ |
| *Elymus triticoides* Buckl | ........ | ........ | — |
| *Elymus virginicus* L | — | — | ........ |
| *Hordeum caespitosum* Schribn | ........ | ........ | — |
| *Hordeum gussoneanum* Parl | ........ | ........ | — |
| *Hordeum jubatum* L | — | — | — |
| *Hordeum pusillum* Nutt | — | — | ........ |
| *Hordeum murinum* L | ........ | ........ | — |
| *Hordeum vulgare* L | ........ | — | ........ |
| *Hystrix Hystrix* (L.) Millsp | — | — | ........ |
| *Notholcus lanatus* (L.) Nash | ........ | ........ | — |
| *Secale cereale* L | ........ | † One uredinium. | ........ |
| *Sitanion Hystrix* (Nutt.) J. G. Sm | ........ | — | ........ |
| *Triticum aegilops* Beauv | ........ | ........ | † Few. |
| *Triticum aestivum* L | † Many. | † Many. | † Many. |

— No infection. † Uredinia produced.

Except for the one uredinium produced on *Secale cereale*, *Triticum aestivum* and *T. aegilops* were the only species infected.

## DESCRIPTION OF AECIA

The following description has been drawn from the aecia obtained in the cultures discussed above.

Pycnia amphigenous, mostly epiphyllous, numerous, crowded upon more or less swollen reddish brown to yellowish areas 2 to 15 mm. in diameter, conspicuous, subepidermal, honey-yellow, globoid or flattened globoid, 80 to 145 μ broad by 80 to 130 μ high; ostiolar filaments 95 to 190 μ long, agglutinated to form a prominent, broad column.

Aecia hypophyllous, crowded in more or less swollen, gall-like, reddish brown or yellowish areas 2 to 15 mm. in diameter, cupulate or short cylindric, 0.2 to 0.6 mm. in diameter, up to 0.5 mm. high; peridium white or yellowish, the margin erose or somewhat lacerate, recurved; peridial cells oblong or somewhat rhomboidal in longitudinal radial section, 14 to 19 by 18 to 29 μ, abutted or slightly overlapping, the outer wall 6 to 7 μ thick, transversely striate, the inner wall thinner 2 to 3 μ, very coarsely verrucose; aeciospores angularly globoid or ellipsoid, 16 to 20 by 16 to 26 μ; wall colorless, thin, 1 μ or less, very closely and finely verrucose.

The pycnia and aecia usually were produced in definite galls or swellings. These galls apparently were formed by the excessive enlargement of the cells of the infected areas, especially those of the mesophyll. When infection took place in the young, rapidly developing tissue of the petiole, galls developed (Pl. 21, D) which were 10 or 15 times as large as the normal petiole. A very noticeable odor, resembling that of the hyacinth, was often detected as the pycnia reached maturity.

## GENERAL DISCUSSION OF RESULTS

The discovery that species of Thalictrum are the aecial hosts for *Puccinia triticina* goes to support Arthur's contention (*1, v. 9, p. 304*) that the leafrust of wheat is closely related to grass rusts of the type of *Puccinia Agropyri*, having aecia on species of the family Ranunculaceae. A number of cultures have been made with rusts of this type, connecting them with various species of Thalictrum. Plowright, in England (*21, p. 181*), connected aecia on *Thalictrum flavum* with a rust on *Agropyron repens*. To this rust he gave the name *Puccinia persistens* Plowr. He considered *Aecidium Ranunculacearum* γ *Thalictri flavi* DC., and *Aecidium Thalictri flavi* (DC.) Winter as synonyms, and describes the aecia as occurring on thickened spots with aeciospores subglobose 17 to 20 by 20 to 30 μ. Fischer (*12, p. 57–63*), in Switzerland, cultured a rust from *Poa nemoralis* var. *firmula* on *Thalictrum minus*, *T. aquilegifolium* and *T. foetidum*. On account of the morphological similarity, he concluded that his material belonged to *Puccinia persistens*, although he made no cultures on either *Agropyron repens* or *T. flavum*. He describes the aecia (*13, p. 347–349*) as having peridial cells with the outer wall 4.5 to 13.5 μ thick and the inner 2 to 6 μ and aeciospores 10 to 21 μ broad and up to 28 μ long. An examination of Sydow's Uredineen No. 725, issued as *Puccinia persistens* on *T. aquilegifolium*, shows the following measurements: Peridial cells, 18 to 23 by 21 to 26 μ; outer wall, 7 to 9 μ; inner, 3 to 5 μ; aeciospores, 16 to 19 by 19 to 26 μ.

Juel (*16, p. 411*), in Sweden, made cultures connecting aecia on *Thalictrum alpinum* with a rust on *Agrostis borealis* and *Anthoxanthum odoratum*. To this rust he gave the name *Puccinia borealis* Juel, and considered *Aecidium thalictri* Grev. as a synonym. His description follows: Pycnia not present; aecia not causing hypertrophy of host tissue; aecio-

spores about 13 μ in diameter. The Sydows (*23, p. 718–719*) give the measurement of the aeciospores as 13 to 18 μ in diameter or 13 to 16 by 18 to 20 μ and note that no swellings are produced on the leaves of the host. An examination of Eriksson's Fungi Parasitici Scandinavici 432a, collected by Juel in Norway, gives the following measurements: Peridial cells, 16 to 19 by 19 to 29 μ; the outer wall, 10 μ; the inner, 3 to 4 μ; aeciospores 14 to 16 by 16 to 21 μ.

Rostrup (*22, p. 269–273*), in Denmark, obtained infection with aeciospores from *Thalictrum minus* on *Elymus arenarius* and considered the rust to be *Puccinia Elymi* Westendorp. The writers have seen neither description nor material of these aecia.

The Sydows (*23, p. 827*) mention that Lindroth in Finland connected an aecidium on *Thalictrum majus* with a rust on *Agropyron caninum*. No description or material of this connection is available for study.

In North America a number of connections have been established by the cultures of Arthur and of Fraser. Arthur (*1, v. 1, p. 248–249*) reports culturing a rust found associated with aecia on *Thalictrum sparsiflorum* from *Bromus Porteri* to *T. dioicum*. To this he gave the name *Puccinia alternans*. He describes the aecia as having peridial cells 21 to 29 μ long with the outer wall 9 to 12 μ thick and the inner 5 to 7 μ and with aeciospores 15 to 20 by 17 to 24 μ. A number of other species of Thalictrum are given as hosts.

Arthur (*1, v. 2, p. 226*) also reports obtaining infection from telia on Agropyron resulting in aecia on *Thalictrum alpinum* but not on *T. dioicum*. This material he considered as belonging in *Puccinia obliterata* Arth., which he had previously shown as having aecia on Aquilegia. A study of the material obtained by this culture shows little or no hypertrophy of the host tissue. The peridial cells measure 16 to 21 by 24 to 32 μ, having the outer wall 7 to 9 μ thick and the inner 3 to 5 μ. The aeciospores measure 14 to 18 by 18 to 23 μ.

Still another connection was obtained by Arthur (*1, v. 8, p. 132–133*) when he cultured a rust on *Festuca Thurberi* to *Thalictrum dioicum*, producing aecia. To this he later (*2, p. 113*) gave the name *Puccinia Cockerelliana* Bethel. He gives the peridial cells as 16 to 23 by 27 to 36 μ with the outer wall 6 to 8 μ and the inner 2 to 3 μ and aeciospores 18 to 24 by 20 to 29 μ with a wall 1.5 to 2.5 μ thick. The natural host for the aecia is given as *T. Fendleri*.

Fraser (*14, p. 131–133*) reports sowing aeciospores from *Thalictrum dasycarpum* on *Elymus canadensis*, *E. virginicus*, *Agropyron tenerum*, *A. Richardsonii*, *Hordeum jubatum*, *Triticum vulgare*, and *Bromus ciliatus*, obtaining infection on *E. canadensis*, *E. virginicus*, *H. jubatum*, and *B. ciliatus*. When, however, the rust obtained upon *B. ciliatus* was sown on *E. virginicus*, *A. tenerum*, *A. Smithii*, *A. repens*, and *H. jubatum* no infection was obtained on these species. From these results Fraser concludes that two strains of *Puccinia Agropyri* Ellis and E.,

were present in the aecial material on Thalictrum which he used for the culture. A study of the aecia used in these cultures shows the dimensions of the peridial cells to be 15 to 19 by 23 to 29 $\mu$ with the outer wall 7 to 10 $\mu$ and the inner 3 $\mu$ thick and the aeciospores 14 to 19 by 19 to 23 $\mu$ in diameter.

A comparison of the foregoing description of the aecia of *Puccinia triticina* with the measurements given for the various grass rust aecia on Thalictrum shows surprisingly little variation. The aecia of *P. Cockerelliana* show the greatest difference, having larger peridial cells and somewhat larger aeciospores with much thicker walls than the aecia of *P. triticina*. Slightly smaller aeciospores occur in *P. borealis* and *P. obliterata*, and the aecial infection causes little or no hypertrophy of the host The remaining aecia differ mainly in slightly thicker walls of the peridial cells.

It is evident that *Puccinia triticina* is closely related to *P. persistens*. Whether the name *Aecidium Thalictri-flavi* (DC.) Wint. should apply to the aecial stage of the former is a question which can not be answered with the available information. De Candolle (*7, p. 97*) described *A. Ranunculacearum* for aecia occurring on the family Ranunculaceae and as a variety of this gives *Thalictri-flavi* without further description. Winter (*24, p. 269*) raises this variety to specific rank and gives a description which, however, could apply to the aecia of either rust. As both *P. triticina* and *P. persistens* are common rusts throughout Europe, there is no way of determining definitely to what aecia the name was applied beyond the fact that they were on *Thalictrum flavum*. As it has been shown that at least some aecia on that host in England belong to *P. persistens* the name *A. Thalictri-flavi* should be retained for the present as a synonym of that species, at least until aecia can be found in Europe upon *T. flavum* which will produce the leafrust of wheat.

Upon their grass hosts these rusts present a somewhat greater variation. They all have uredinia with globoid or ellipsoid urediniospores with a varying number of scattered pores, usually more than six, and with few or no paraphyses. The telia are long, covered by the epidermis, usually with more or less stroma present, and the teliospores are cylindric, more or less flattened at the apex, and with a very short pedicel. *Puccinia Cockerelliana* differs most noticeably from *P. triticina* in that the teliospores are much longer and the telia do not remain entirely covered by the epidermis at maturity. *P. Elymi* differs especially in the thicker and darker walls of the urediniospore and in the longer teliospores which are often many-celled. *P. alternans, P. borealis, P. obliterata*, and *P. persistens* differ but little, mostly in the tinting of the urediniospore wall and a slight variation in pore number.

Although the morphological differences between *Puccinia triticina* and the related rusts discussed above are not great, their biologic specializa-

tion to their hosts is very pronounced. This appears to hold true for the aecial as well as the grass hosts. *P. Cockerelliana* and *P. alternans* go to *Thalictrum dioicum*, and *P. Elymi* to *T. minus*, as aecial hosts, neither of which is a favorable host for *P. triticina*. The rust of *T. dasycarpum*, used by Fraser (*14*) in his cultures, is on a host which was not infected by *P. triticina*. *P. borealis* and *P. obliterula* on *T. alpinum* offer no comparison, as *P. triticina* was not sown on that host. *P. persistens*, as cultured by Fischer on *T. minus* and *T. aquilegifolium*, is upon species of Thalictrum unfavorable for *P. triticina*, while *P. persistens* as originally cultured by Plowright upon *T. flavum* is on the most congenial host for the leafrust of wheat. It is very probable that Plowright and Fischer were working with two distinct biologic strains. Although *T. flavum* appears to be a favorable host for both *P. persistens* and *P. triticina*, and these two rusts are very similar in their morphology, the inability of the latter to infect *Agropyron repens* shows that it is biologically distinct from the former. A study is being made of the relationship of *P. triticina* to grass hosts other than wheat. From the data now at hand, it would appear that, in addition to the grasses listed in Table IV, species of Bromus, Festuca, Agrostis, Poa, and Anthoxanthum are immune from the leafrust of wheat. These results indicate that, as far as its telial host is concerned, *P. triticina* also is biologically distinct from other grass rusts having aecia on Thalictrum.

A similar situation exists in the relationship of *Puccinia triticina* to rusts having aecia upon species of other genera of the Ranunculaceae. Slight morphological differences, such as urediniospore size, wall color, and pore number, exist among the different races producing aecia upon species of such genera as Actaea, Anemone, Clematis, Delphinium, etc. A similar, or perhaps greater, biologic specialization is also to be found among these races. The importance of these morphological characters and biological differences which occur among the members of this group can not be fully determined at present on account of our comparatively limited knowledge of but few races. Any final interpretation must await further study of a greater number of such races. On the basis of our present knowledge, the disposition of *P. triticina* must depend largely upon the species concept held. In Europe there is a tendency among certain students of the rusts to consider as species those rusts showing distinct biologic specialization regardless of the absence of morphological difference. In this country, on the other hand, the general tendency is to include in a single species all closely related forms having but little difference in their morphology. Forms limited to a definite host, or hosts, are considered as races of such species. On the former basis, *P. triticina* would be considered a distinct species comparable to *P. Elymi*, *P. Agropyri*, *P. persistens*, etc., while with the latter concept it would be united with all or part of these, each being considered a race of a

collective species to be designated, according to the limitations of the species concept held and the system of nomenclature used, as *P. Agropyri* E. and E. (*1, v. 9, p. 304*), *P. Clematidis* (DC.) Lagerh., or *Dicaeoma Clematidis* (DC.) Arth. (*3, p. 333–337*).

The close biological specialization of *Puccinia triticina* to wheat is of considerable significance with respect to the bearing it has upon the possible origin of this rust and of wheat itself. Since wheat is an introduced plant, it is logical to assume that a rust showing such close biological specialization to it is also introduced and of foreign origin.

It is generally recognized among students of the rusts that a high degree of host specialization must have been acquired in certain groups of species at a very early stage in the evolutionary history of this group of fungi. It is also recognized that the host is the most important factor in the evolution of highly specialized parasitic fungi. As the higher plants have gradually developed during geological times, their rust parasites have developed with them. It therefore appears reasonable to assume that *Puccinia triticina*, which shows such a high degree of specialization to wheat at the present time, had its origin as a distinct strain comparatively early in the development of the group of grasses from which our cultivated wheats have originated. The original distribution of the rust presumably would coincide with the distribution of the ancestral wheats.

A study of the relative susceptibility of various species of Thalictrum to infection by this rust is of interest in this connection. The four most susceptible species of Thalictrum encountered in this investigation are all of foreign origin. The most susceptible of our native North American species, *Thalictrum dioicum*, does not compare in susceptibility with these four foreign species—*T. flavum*, *T. Delavayi*, *T.* sp. 55, and *T.* sp. 98—but is comparable to the resistant foreign species such as *T. minus*. That these foreign susceptible species of Thalictrum are also to be considered as indicating a foreign origin of the rust would appear to follow if the nature of aecial infection is considered. Heteroecious rusts in most cases infect their aecial hosts only for a comparatively short period of time while the teliospores are germinating in the spring. The infection produced, not being able to propagate itself upon such hosts, causes little or no damage, and they are in most cases soon able to outgrow it. On this account it is hardly to be expected that a natural selection of resistant strains of aecial hosts takes place in nature comparable to that which occurs where the host is killed or prevented from producing seed. Should this occur in heteroecious rusts which are not able to survive adverse conditions in winter or summer by means of urediniospores, such a selection would be fatal to the rust itself. For this reason the susceptibility of the aecial hosts of *P. triticina* may be taken as indicative of its origin. It is true that susceptibility of a host species does not necessarily indicate that such a species was a native host of the rust nor does resistance

of some one species denote that the rust is not to be found in the habitat of such a resistant species, for susceptibility or resistance is not dependent upon the presence or absence of the rust but may develop with the species in any region. It is regarded as significant, however, that of the species of Thalictrum tested the most susceptible are exotic. This fact, taken with the foreign origin of wheat itself, is confirmative of the foreign origin of the rust.

The native habitats of two of these species of Thalictrum are known with some degree of certainty. *Thalictrum flavum* is found throughout Europe, western Asia, and Asia Minor. *T. Delavayi* is given by the Index Kewensis [1] as occurring in western China, probably indicating a distribution in the little-known mountainous regions of Tibet and Chinese Turkestan. These two species, taken together, would therefore indicate as the most probable original distribution a region in which the two Thalictrum species may border or overlap, such as that of southwestern Asia. Such an origin would indicate a like origin for wheat itself, which, we believe, would agree with the latest theories advanced as to the original home of wheat.

Concerning the occurrence and distribution of the aecia of *Puccinia triticina* but little can be said with the data at hand. It is also probable that the aecial stage occurs, and probably assumes greater importance, in other regions than it may in either Europe or North America, where the rust is known to overwinter in its uredinial stage. Thus in such countries as India, where Butler has shown there is no oversummering of the rust, the Thalictrum species of the foothills may be of importance in starting the rust the next season. The question of the rôle which the aecia of the leafrust of wheat plays in its life history and distribution must, however, be left for future research to solve. Whether native species of Thalictrum serve as aecial hosts in North America and, if so, whether they serve as important factors in the development of the leafrust of wheat and whether there is more than one race of the leafrust, as indicated by the results obtained from the Great Plains area, or whether these results were due to other causes, such as climatic or seasonal effects weakening the vitality of the teliospores, are all questions on which further investigation is planned. Other species of Thalictrum from foreign botanic gardens also will be studied in regard to their susceptibility to the orange leafrust of wheat.

## SUMMARY

(1) The aecial stage of *Puccinia triticina* has been produced in greenhouse cultures upon several species of Thalictrum.

(2) The various species of Thalictrum show varying degrees of susceptibility to the rust. *Thalictrum occidentale* was apparently immune. Upon *T. dasycarpum* and *T. polygamum* an occasional devlopment of

[1] INDEX KEWENSIS PLANTARUM PHANEROGAMARUM. SUPPLEMENTUM PRIMUM . . . CONFECERUNT THEOPHILUS DURAND ET B. DAYDON JACKSON. p. 423. Bruxellis 1901–06

pycnia took place. When *T. angustifolium*, *T. aquilegifolium*, *T. dioicum*, *T. minus*, *T. minus adiantifolium*, and *T. polycarpum* were inoculated usually only pycnia resulted, with an occasional weak development of aecia, while in other cases no infection occurred. Two undetermined species of Thalictrum, as well as *T. Delavayi* and *T. flavum*, when inoculated, showed a vigorous development of aecia, increasing in susceptibility in the order named.

(3) *Puccinia triticina* is apparently limited to species of the genus Thalictrum, no infection being obtained upon species of Aconitum, Actaea, Anemone, Aquilegia, Cimicifuga, Clematis, Delphinium, Echium, Hepatica, Hydrophyllum, Impatiens, Mertensia, Myosotis, Ornithogalum, Phacelia, Camassia, Ranunculus, or Trollius.

(4) On account of the morphology and host relationships, *Puccinia triticina* is considered to be very closely related to *P. persistens*, *P. borealis*, *P. alternans*, *P. obliterata*, *P. Elymi*, and *P. Agropyri*, but is separable from these rusts by its sharp biologic limitation to wheat.

(5) *Puccinia triticina* is considered to be of foreign origin, because wheat, for which it shows close specialization, is an introduced host, and because the most susceptible species of Thalictrum which serve as aecial hosts also are exotic.

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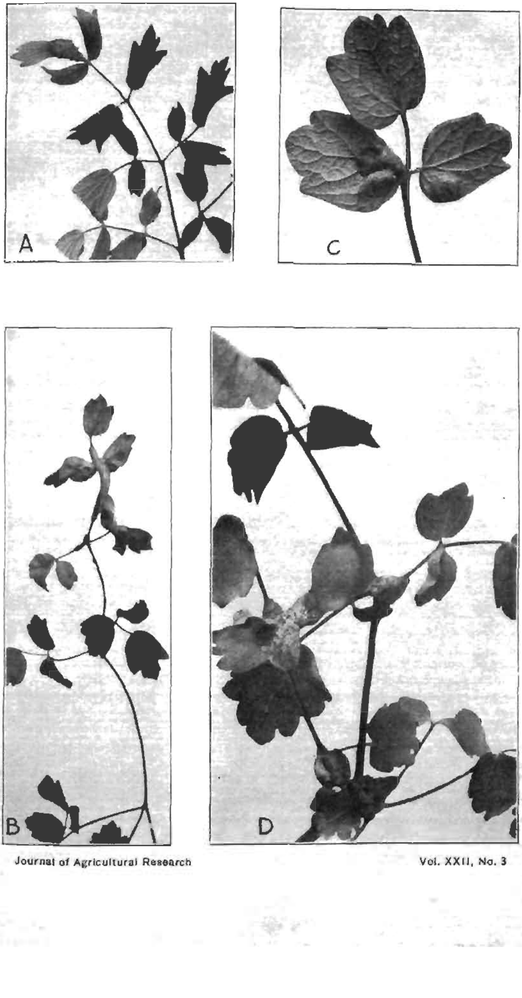
## PLATE 21

A.—Infection produced upon *Thalictrum* sp. (98) inoculated with *Puccinia triticina* from Colton, Wash. (16519).

B.—Infection produced upon *Thalictrum* sp. (98) inoculated with *Puccinia triticina* from Canton, Miss. (8719).

C.—Infection produced upon *Thalictrum flavum* (53) inoculated with *Puccinia triticina* from Hickory, N. C. (7219).

D.—Infection produced upon *Thalictrum* sp. (55) inoculated with *Puccinia triticina* from Hickory, N. C. (7219).


A
C
B
D

# A TRANSMISSIBLE MOSAIC DISEASE OF CHINESE CABBAGE, MUSTARD, AND TURNIP

By E. S. SCHULTZ

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In the fall of 1919, while the writer was selecting different kinds of plants for inoculation experiments with mosaic of Irish potatoes (*Solanum tuberosum* Linn.), Dr. W. A. Orton called his attention to mottling in plants of Chinese cabbage (*Brassica pekinensis* (Lour.) Gagn.), mustard (*B. japonica* Coss.), and turnip (*B. rapa*, Linn.). The mottling resembled that of mosaic plants of other species, such as potato and tobacco. Diseased and healthy individuals were found in the same plot; the former appeared in groups in some parts of the field, suggesting an infectious character of this malady. Evidence bearing upon the nature of this disease, its symptoms, and means of transmission is presented in this paper.

## SYMPTOMS

Mosaic of Chinese cabbage, mustard, and turnip produces a distinct mottling of the leaves, very similar to that of mosaic diseases of the Solanaceae. This mottling is produced by the appearance of irregular light green and dark green areas on the leaves (Pl. B; 22, D, E; 24, A, B). These light green areas usually adjoin the veins, from which they may extend so as to include a considerable area of the leaf surface between the veins. Another very common macroscopic symptom of this disease is the characteristic ruffling and distorting of the leaf surface (Pl. 24, A, B). On the raised areas the dark green patches appear. The leaf margins frequently are much more irregular than in healthy plants, causing some of the leaves to appear somewhat unsymmetrical. In addition to these common abnormalities on the leaves the entire plant may be dwarfed, and the flower stalk and number of blossoms may be considerably reduced (Pl. 22, B; 23, B).

## OCCURRENCE IN THE FIELD

Since mosaic individuals appeared among Chinese cabbage, mustard, and turnip plants growing in adjoining plots, interspecific susceptibility was suggested. Furthermore, it was found that a large percentage of the plants were infested with aphids, *Myzus persicae* Sulz.,[1] one of the

[1] Identified by Dr. A. C. Baker, Entomologist, Bureau of Entomology, United States Department of Agriculture.

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casual agents in the transmission of mosaic and leafroll of Irish potato.[1] In view of these field observations experiments on this disease were conducted in the greenhouse at Washington, D. C., during the winters of 1919–20 and 1920–21.

## TRANSMISSION WITH PLANT JUICE

Chinese cabbage, mustard, and turnip plants showing mosaic mottling were taken from the field and planted in pots in the greenhouse. Only a small percentage of these mature and mosaic plants survived transplanting, so that the supply of mosaic material for inoculations was thus considerably reduced, and therefore only a small number of healthy plants were inoculated at one time.

Inoculations with juice were made by rubbing the leaves between the fingers so that considerable sections of the leaflets were crushed, apparently permitting the applied juice to be absorbed by such areas of the leaf as still remained free or partly free from mutilation. Such operations were performed chiefly upon the youngest leaves, the first applications being made when the plants had developed about five leaves. In Table I the results of these inoculations are presented.

TABLE I.—*Inoculations with juice from mosaic plants*

| Variety and species inoculated. | Time of inoculation. | Source of juice. | Number of plants inoculated. | Number of plants mosaic.[a] | Per centage mosaic. |
|---|---|---|---|---|---|
| Southern Prize turnip | Dec. 4, 1919 | Mosaic Southern Prize turnip. | 9 | 6 | 67 |
| Do | do | Healthy turnip | 9 | 0 | 0 |
| Do | Dec. 6, 1919 | Mosaic Green Mountain potato. | 5 | 0 | 0 |
| Mustard | do | do | 5 | 0 | 0 |
| Do | Mar. 8, 1921<br>Mar. 21, 1921 | do | 6 | 0 | 0 |
| Do | Mar. 9, 1921<br>Mar. 21, 1921 | Mosaic mustard. | 8 | 5 | 63 |
| Do | Jan. 15, 1921 | Mosaic pe-tsai or Chinese cabbage. | 5 | 3 | 60 |
| Pe-tsai or Chinese cabbage. | Dec. 4, 1919 | do | 8 | 6 | 75 |
| Do | do | Healthy | 5 | 0 | 0 |
| Do | Dec. 6, 1919 | Mosaic Green Mountain potato. | 4 | 0 | 0 |

[a] Date of last observation April 2, 1920 and 1921.

The data indicated in Table I disclose the fact that mosaic mottling was obtained only when juice from a mosaic plant was introduced into

[1] SCHULTZ, E. S., FOLSOM, Donald, HILDEBRANDT, F. Merrill, and HAWKINS, Lon A. INVESTIGATIONS ON THE MOSAIC DISEASE OF THE IRISH POTATO. *In* Jour. Agr. Research, v. 17, no. 6, p. 247–274, pl. A–B (col.), 25–30. 1919. Literature cited, p. 272–273.

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a plant of the same or a closely related species.[1] No mosaic mottling appeared on any of the cruciferous plants inoculated with juice from mosaic potato. With a more adequate supply of crucifer mosaic material and repeated applications it is probable that every plant treated would have developed mosaic mottling, such as has frequently been obtained with mosaic potato juice inoculations on the Irish potato.[2]

The first mosaic mottling was observed from 20 to 30 days after inoculation, which also corresponds very closely with the incubation period for mosaic of Irish potato. The results in Table I also disclose successful inoculations on plants in different species of Brassica. Further evidence on this interspecific infection is presented in Table II on transmission by means of aphids.

## TRANSMISSION WITH APHIDS

Since aphids were found on every mosaic plant examined in the field and on account of the fact that these insects have been found to transmit mosaic of tobacco,[3] spinach blight,[4] and mosaic of potato,[5] experiments were carried on with these insects. Aphids belonging to *Myzus persicae* Sulz. were used in this investigation. These insects were originally collected from the morning-glory and transferred to healthy turnip and mustard plants on which they were cultured while confined under cages until needed for inoculation. Neither the morning-glory nor the turnip or mustard plants on which these insects fed before being transferred to mosaic Chinese cabbage and turnip developed mosaic mottling. This indicates that the morning-glory apparently was free from mosaic, at least from the type which could infect the crucifers used in this experiment.

When the healthy plants for inoculation had developed from five to eight leaves, aphids were transferred from the cultures to mosaic plants, where they were allowed to feed for a few days before they were introduced to the healthy plants. All inoculated plants also were confined in cages so as to prevent dispersal from one species to another. After the aphids had fed from 7 to 14 days on the inoculated plants they were killed by tobacco fumigation in a fumigation chamber. These plants were now allowed to grow without cages in a greenhouse where fumigation was practiced at regular intervals for the control of aphids. Since mosaic mottling developed from 12 to 30 days after these insects were killed by fumigation, mosaic mottling can not be attributed simply to the mechanical injury produced by the aphids. This fact is further

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[2] SCHULTZ, E. S., FOLSOM, Donald, HILDEBRANDT, F. Merrill, and HAWKINS, Lon A. OP. CIT.

[3] ALLARD, H. A. THE MOSAIC DISEASE OF TOBACCO. U. S. Dept. Agr. Bul. 40, 33 p., 7 pl. 1914.

[4] MCCLINTOCK, J. A., and SMITH, Loren B. TRUE NATURE OF SPINACH-BLIGHT AND RELATION OF INSECTS TO ITS TRANSMISSION. *In* Jour. Agr. Research, v. 14, no. 1, p. 1-60, pl. A (col.), 1-11. 1918.

[5] SCHULTZ, E. S., FOLSOM, Donald, HILDEBRANDT, F. Merrill, and HAWKINS, Lon. A. OP. CIT.

confirmed by the control plants which remained free from mosaic mottlin[g] after aphids taken from healthy plants had fed upon them. The result[s] secured from inoculation with aphids are presented in Table II.

TABLE II.—*Transmission of mosaic of mustard, pe-tsai, and turnip by means of aphid[s]*

| Variety inoculated. | Date of inoculation. | Approximate number of aphids transferred. | Source of aphids. | Number of plants inoculated. | Date of first symptoms. | Number of plants mosaic.[a] | Percentage mosaic. |
|---|---|---|---|---|---|---|---|
| Southern Prize turnip | Jan. 12, 1920 | 50 | Mosaic Southern Prize turnip. | 2 | Feb. 2, 1920 | 2 | 10 |
| Do | Jan. 26, 1920 | 50 | ...do | 2 | Feb. 20, 1920 | 2 | 10 |
| Do | Jan. 12, 1920 | 50 | Healthy turnip | 3 | ...... | 0 | 0 |
| Purple Top turnip | Jan. 22, 1920 | 12 | Mosaic Southern Prize turnip. | 3 | Feb. 14, 1921 | 3 | 10 |
| Seven Top or South-Prize turnip. | Feb. 24, 1920 | 100 | Mosaic mustard | 2 | Mar 20, 1920 | 2 | 10 |
| Do | Mar. 5, 1920 | 100 | ...do | 2 | Mar. 31, 1920 | 2 | 10 |
| Do | Mar. 10, 1920 | 50 | ...do | 1 | Apr. 2, 1920 | 1 | 10 |
| Mustard | Jan. 12, 1920 | 50 | ...do | 1 | Feb. 11, 1920 | 1 | 10 |
| Do | Jan. 19, 1920 | 100 | ...do | 2 | Feb. 19, 1920 | 2 | 10 |
| Do | ...do | 100 | Healthy mustard | 1 | ...... | 0 | 0 |
| Do | Jan. 26, 1920 | 50 | Mosaic mustard | 2 | ...... | 2 | 10 |
| Do | ...do | 50 | Healthy mustard | 1 | ...... | 0 | 0 |
| Do | Mar. 12, 1921 | 50 | Mosaic mustard | 5 | Apr. 2, 1921 | 5 | 10 |
| Do | Jan. 29, 1921 | 25 | Mosaic Southern Prize turnip. | 3 | Feb. 20, 1921 | 3 | 10 |
| Do | Feb. 19, 1921 | 50 | ...do | 5 | Mar. 25, 1921 | 2 | 4 |
| Do | Feb. 8, 1921 | 25 | ...do | 2 | ...... | 2 | 10 |
| Do | Jan. 15, 1921 | 12 | Mosaic Chinese cabbage. | 3 | Feb. 14, 1921 | 3 | 10 |
| Do | Jan. 20, 1921 | 25 | ...do | 1 | Feb. 20, 1921 | 1 | 10 |
| Do | Feb 19, 1921 | 25 | ...do | 5 | Mar. 28, 1921 | 3 | 6 |
| Do | Mar 2, 1921 | 50 | Mosaic Green Mountain potato. | 5 | ...... | 0 | 0 |

[a] Date of last observation, Apr 2, 1920 and 1921.

From the data indicated in Table II it is evident that aphids transmit mosiac of the crucifers in question between different species as well a[s] among plants of the same species, as was suggested in Table I on juic[e] inoculations by means of rubbing. It will also be noted that the period in which the first mosaic mottling appeared corresponds very closely t[o] that obtaining with mosiac diseases of other plants. As with the plant[s] inoculated by rubbing, the plants inoculated by means of aphids developed the mosaic symptoms only on the younger leaves. Mosaic symptoms on the inoculated plants were like those which were observed on mosaic lots taken from the field.

Since turnips from mosaic plants taken from the field continued t[o] produce mosaic foliage it is apparent that such plants become a sourc[e] of infection if planted near susceptible varieties. Mustard seed fro[m] mosaic mustard plants apparently develop healthy seedlings. This wa[s] observed in 100 seedlings, which were grown from seed from mosai[c] mustard in the fall of 1920; in this test every seedling was free fro[m] mosaic mottling.

SUMMARY

From these preliminary observations and experiments it appears that e crucifers here mentioned may be added to the list of plants suscepti- ; to mosaic, a disease whose cause has not been discovered but which n be transmitted from mosaic to healthy plants by direct transfer of ce as well as by means of aphids which apparently are very effective tural agents in the dissemination of this disease.

## PLATE B

1.—Leaf from healthy turnip, control to mosaic turnip in figure 2.

2.—Leaf from mosaic turnip, mosaic induced by aphids transferred from mosaic turnip plant.

3.—Leaf from healthy mustard, control to mosaic mustard in figure 4.

4.—Leaf from mosaic mustard, mosaic produced by aphids from mosaic mustard.

3
4
2
1

A
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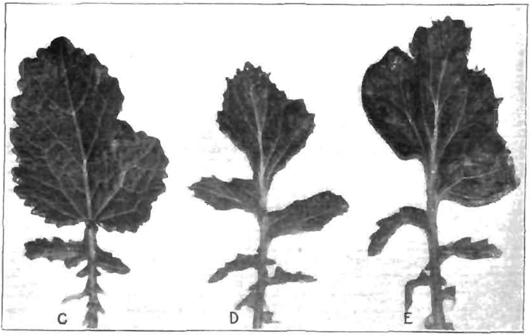
C
D
E

PLATE 22

A.—Healthy turnip plant, control to B. Aphids from healthy turnip were allowed to feed on this plant. Planted the same time as B.

B.—Mosaic on turnip plant, variety Seven Top or Southern. Mosaic mottling appeared 26 days after the introduction of aphids from a mosaic Southern turnip plant.

C.—Leaf from A, healthy.

D, E.—Two mosaic leaves from B. Mosaic mottling and ruffling apparent on the diseased leaves.

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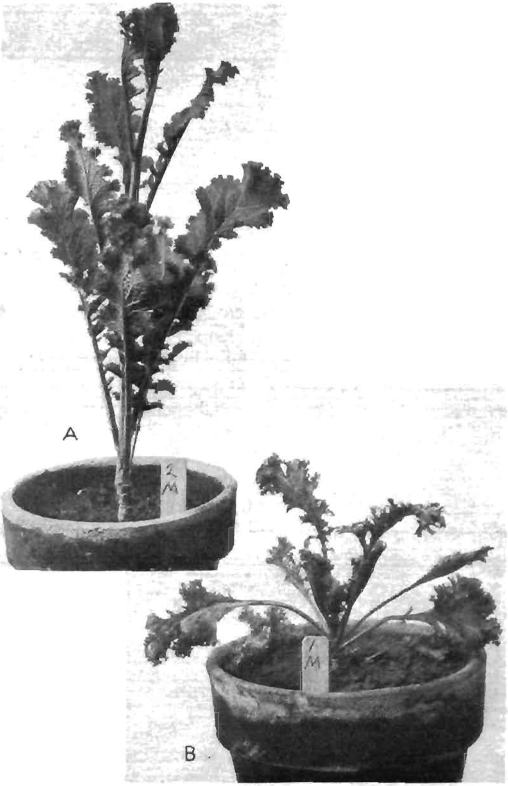
PLATE 23

A.—Healthy mustard plant, control to B. Planted the same time as B.

B.—Mosaic on mustard plant produced by transferring aphids from mosaic mustard. Distinct mosaic mottling was noted 28 days after introduction of aphids.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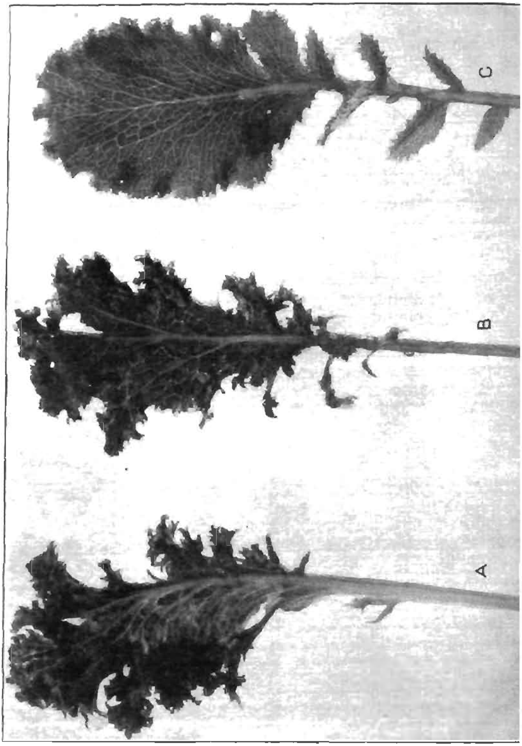
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# JOURNAL OF AGRICULTURAL RESEARCH

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## FLORA OF CORN MEAL

By CHARLES THOM, *Mycologist in Charge*, and EDWIN LEFEVRE, *Scientific Assistant, Microbiological Laboratory, Bureau of Chemistry, United States Department of Agriculture*

### INTRODUCTION

Corn meal as it comes from the mill carries the mycelia of certain fungi which infect unground grain. In addition, numerous species of molds and bacteria, present in spore form as contaminations upon the surfaces of sound kernels or as saprophytes in partially spoiled grains, are recoverable by routine cultural examination of the finished meal. Many experiments, extending over several years and including the work of various members of the Microbiological Laboratory, show that certain groups of organisms are practically always abundant in such cultures. Other species are usually present, but in smaller numbers, and many forms are obtained occasionally as accidental contaminations. In undertaking to study this complex flora, it may be possible to determine by routine culture the species represented and something of their relative abundance in the sample, but the list so obtained gives little information as to the relative importance of the individual species as causes of spoilage in the product.

The culture media commonly used in such routine examination of foodstuffs present conditions for the growth of microorganisms which differ greatly from those found in corn meal. The nutrients used in preparing such media are selected because they are readily assimilable to most organisms. These nutrients appear in solution or in jelly-like masses which contain high percentages of moisture. Corn meal, on the other hand, presents a range of composition, according to Winton and his associates (*8*),[1] approximately as follows: Moisture, 10 to 18 per cent, but under usual commercial practices ranging from 12 to 15 per cent; protein, 5 to 10 per cent; fat, 1 to 5 per cent, according to the method of milling; nitrogen-free extract, including starch and sugar, 68 to 78 per cent. Of the nitrogen-free extract, sugars constitute perhaps 3 per cent, and gums and dextrin, some of which are readily fermentable, perhaps an equal quantity. In dealing with this product as a substratum for organisms, the percentage of water found is an important limiting

[1] Reference is made by number (italic) to "Literature cited," p. 188

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factor. Obviously this product, even at its maximum moisture content, presents a marked contrast to laboratory media as usually prepared. Nevertheless, corn meal has been so often found an unstable product that it is commonly milled only for consumption within a few weeks or by methods intended to eliminate the most readily fermentable portions of the grain.

Under ordinary conditions of handling, spoilage in this product appears in one of the following forms: Souring, rancidity, mustiness, the formation of clumps or balls, extensive concretions which may involve the solidification of an entire bag, or the formation of a hard, cylindrical outer mass with the center loose and mealy. Heating occurs only in the wettest samples. Much corn meal, if held beyond a very short period, develops a musty, moldy, or sour odor and shows occasional balls or masses of meal held together by mold, which bring about losses in palatability and market quality in the product. Such changes as rancidity and the formation of extensive concretions into moldy masses are so obviously due to high moisture content and involve such losses that they have been almost eliminated from commercial practice. When losses occur the meal is found to carry more than a critical moisture percentage. This may be due either to milling corn which is insufficiently dried or to the storage of the meal under conditions which will maintain a moisture content above the danger point. For the samples used in all series reported here this figure was approximately 13 per cent (2).

## CULTURAL EXAMINATION

In routine cultural examination reported here, plain agar was used for bacterial counts, wort agar for mold counts, and dextrose-litmus shake agar to determine acid, gas, and anærobic growth. The presence of particular organisms was determined by the use of special methods on special media. Experimentation covered a range wide enough to justify the restriction of routine cultures to the media already noted.

After comparative study of many series of cultures, Table I is introduced as giving a group of cultural results fairly typical for commercial meal in sound, merchantable condition. The nine samples reported were purchased in different retail stores of Washington, D. C., during October and November, 1920. Four of them were yellow and fairly coarsely ground. The white meals were softer or more finely ground. All were bolted. All showed by microscopic examination traces of both bran and germ, although these portions were scanty in certain samples. The history of the samples was not obtained.

These samples were sound in appearance and odor. There was no evidence of the multiplication of microorganisms. Among the bacterial colonies micrococci, members of the mesentericus and of the colon-aerogenes groups were characteristically present. Special tests in cab-

bage juice showed, in four of the nine samples, the presence of lactobacilli with the morphology and cultural characters of the organism of pickle and sauerkraut fermentation. No bacterial colonies were obtained in plain agar from two of the samples. A duplicate of sample 9 proved equally negative. Mold colonies were obtained in all samples. These represented in varying proportions *Aspergillus repens* De Bary, *A. niger* Van Tieghem, *A. flavus* Link, Fusarium, various mucors, and unidentified colonies.

TABLE I.—*Results of cultural examination of commercial corn meals*

| Sample No. | Bacteria per gram on plain agar | Molds per gram on wort agar. | Bacteria per gram on dextrose-litmus agar. | Acid colonies. |
|---|---|---|---|---|
| | | | | *Per cent.* |
| 1 | 10,000 | 10,000 | 16,000 | 50 |
| 2 | 5,000 | 1,000 | ........ | ........ |
| 3 | 55,000 | 12,000 | 42,000 | 60 |
| 4 | 60,000 | 10,000 | 13,000 | 50 |
| 5 | 70,000 | 400,000 | 10,000 | 50 |
| 6 | 5,000 | 20,000 | 8,000 | 60 |
| 7 | ........ | 3,000 | ........ | ........ |
| 8 | 10,000 | 11,000 | 4,000 | 100 |
| 9 | | 5,000 | 3,000 | 30 |

A more extensive series of studies was conducted in cooperation with the Plant Chemical Laboratory of this bureau. The general results of this experiment are described elsewhere (2). In brief, during the spring of 1920, a series of bags of meal were prepared for this storage experiment from corn bought by the mill in the regular course of business. This grain, while sold as No. 2, was obviously wet and barely passable as a fair product. Infected and even badly decomposed ears were not uncommon among the ears of corn received in bulk. Although the lots of meal included were milled at water contents varying from 12.7 to 16.18 per cent, the conditions of storage were such that no spoilage determinable by the senses took place. Cultures were made from the meal as freshly ground in April, then, beginning May 5, once each week until July. In all these cultures no evidence of multiplication of either mold or bacteria was found. It was, therefore, possible to follow the relative numbers of viable organisms in the various groups from the time of grinding through the four months of storage.

In the freshly milled samples the average count of colonies of bacteria upon plain agar was about 1,000,000 per gram of meal, with variations from 600,000 to 1,600,000. Upon wort agar the count of mold colonies averaged about 100,000 per gram of meal, with variations in different samples from 70,000 to 160,000. Of the bacterial colonies observed about 60 per cent were acid producers.

For comparison a special series of samples were prepared by adding 5 per cent of meal made from corn markedly rotted with Diplodia and Fusarium. In the freshly ground meal of this series the bacterial count upon plain agar was about 2,600,000. The count of mold colonies upon wort agar was about 110,000. About 70 per cent of the bacterial colonies were acid producers.

After storage for approximately one month (May 20 and 21) samples from a particular lot of five bags of the regular meal showed an average count of 108,000 bacterial colonies and 15,000 molds. Samples from the same bags on June 30 showed an average count of 12,600 bacterial colonies, and 7,600 mold colonies. Without placing emphasis upon exact figures, these cultural results are fairly typical of the mass of figures obtained from cultures made weekly from representative samples involving the whole series of 88 bags of meal. These figures are readily comparable with those obtained from commercial samples (Table I). Discrepancies which occur may perhaps be accounted for by the fact that samples 3, 4, and 5 were evidently the product of local mills, sold fairly quickly after milling, while samples 2, 7, and 9 were clearly the product of special processes and handled under conditions involving much slower distribution.

In this lot of meal, therefore, the conspicuous change due to storage was the drop in the number of viable organisms to about 1 per cent of the original number of bacteria and perhaps 10 per cent of the original number of molds. The larger part of this decrease occurred during the first six weeks, with a slow reduction throughout the succeeding periods.

In connection with the study of these figures, data obtained by Thom and Stiles (unpublished) in examining Winton's (*8*) samples[1] in 1914 were restudied and compared with the results here considered. Winton's corn meal varied in initial moisture content from 19.27 to 10.79 per cent. In those lots of meal (A, B, and C) carrying moisture markedly above 13 per cent, the evidence of multiplication of molds and bacteria was clearly discernible. Musty odors and balls of meal held together by mold were present in every sample. In cultures, the count of colonies of molds and bacteria reached 13 million in the wettest lot. Of these several million were *Aspergillus flavus*. The predominant organisms were molds rather than bacteria, but there was fairly clear evidence of some bacterial multiplication at the higher water percentages.

In the roller-ground samples of lots D, E, F, which did not spoil and whose water percentage was near to or less than 13, the total counts found by Stiles approached very nearly those already given in this paper. These examinations began too late in the storage period to show that part of the bacterial flora which dies off rapidly. The stored samples still showed some acid organisms, but micrococci and aerobic spore

[1] Samples of the meal studied were examined bacteriologically by G. W. Stiles, formerly of the Bureau of Chemistry, and for mold activity by Charles Thom, then in the Bureau of Animal Industry (*8, p. 25*).

formers of the mesentericus group formed the majority of the bacteria obtained.

In the lots with moisture content decreasing toward 13 per cent there was progressive reduction in the number of active species of molds. Extensive experimentation showed clearly that *Aspergillus repens* was the agent which formed the balls of meal loosely held together with mold hyphae, which characterized meal containing barely enough water to start spoilage. In another series of experiments *A. flavus* began to be active only in samples containing about 16 per cent of water. Yeasts, mucors, and Penicillia were reported by Stiles only in the sample carrying about 19 per cent of water.

During the examination of the preliminary samples in the 1920 experiment, an effort was made to identify the groups or actual species represented. As a matter of routine, inoculations were made from each flask prepared for diluting plates (consisting of 5 gm. of the meal to 45 cc. of sterile water) into the following media: Plain milk, gelatin, and litmus lactose broth. Smears were also made on Endo's agar in each instance. In every case there was prompt coagulation of the milk, with extrusion of whey, but no digestion of curd. Pink rings formed near the surface. Gelatin was liquefied in every instance, and acid and gas formed in all broth tubes. Growth in Endo's media indicated the presence of *Bacterium aerogenes* Escherich. Further cultural studies showed that *Bact. aerogenes* was the predominant bacterial species present in all these samples. This predominance was maintained throughout the series of examinations made. Microscopical examinations of smears made in each case, however, showed the presence of spore-bearing bacteria, especially the mesentericus group, and micrococci of various kinds. Dextrose agar tubes often contained colonies growing deep in the media, indicating the presence of anaerobic bacteria. Yeasts were found in all samples, their growth being largely of the mycoderma type. The plates showed many mold colonies. Various mucors, species of Fusarium, *Aspergillus flavus*, *A. niger*, and occasional green Penicillia were observed. The species of molds present on the plates varied from period to period and with the sample. Molds were always more numerous on plates made from meal to which Fusarium and Diplodia had been added, but growth on these plates did not show dominance of these particular forms.

Evidence of the effect of bolting upon the abundance of organisms was furnished in the 1920 experiment by the examination of samples of two series of five bags each, representing a single lot of meal, one-half of which was bolted and the other half unbolted. The bolting to which these samples were subjected removed a considerable part of the bran but little of the germ from the meal. After one month of storage, the bolted meal showed an average of 34,000 bacterial colonies and 20,000 mold colonies. The unbolted samples showed 108,000 bacterial colonies

and 15,000 molds. This observation was confirmed by a restudy of Stiles's unpublished examination of Winton's (*8*) samples. Of every lot of corn handled, part was ground in a stone mill without sifting or bolting and part was carefully "degerminated" and "roller" ground. In the bolting process all of the bran was taken out, and many of the samples consisted almost completely of horny endosperm. In that part of this series made up of meals in which no multiplication of microorganisms occurred, bolting consistently reduced the cultural count of microorganisms below that of the stone-ground meal. Frequently the number found in the bolted meal was less than one-tenth of that in the stone-ground meal.

By removing the bran, bolting takes away the largest area of contamination with saprophytic organisms. The tip of the kernel and the germinal area carry the majority of the infections found in corn. Study of many samples of corn over a period of years shows that invasion of the germinal area by molds is not uncommon in corn which has not been fully matured or has not been promptly and thoroughly dried. Samples have frequently shown the invasion of the germ in every kernel by *Aspergillus repens*. Recently samples representing a bulk shipment have shown nearly every grain to contain one or the other of two species of Penicillium. Meal therefore may be so milled and sifted or bolted as to remove the larger part of all contaminations, as well as those mold infections which do not involve general disintegration. The cleaning process before milling removes the grains thoroughly rotted by Fusarium and Diplodia. Corn has still been seen going into the rolls of a mill in which the low grade of the stock could not have been concealed if it had passed through a stone mill without being bolted. The product, however, was going into human food without showing tangible evidence of the low quality indicated by the unground grain. In other words, the fractional milling of low-grade grain makes possible such separation as turns the infected portions of the grain into oil stock or cattle feed and the solid or horny portions which are less obviously damaged into meal.

The literature of maize deterioration is reviewed by Alsberg and Black up to 1913 (*1*). The activity of Fusarium and Diplodia as causes of rotting in ear corn was discussed by Burrill and Barrett (*3*) and that of Diplodia alone by Heald, Wilcox, and Pool (*4*).

More recently McHargue (*6*) has studied the activities of certain fungi and their relation to commercial conditions in the handling of the product. Excessive moisture in the grain is regarded as the limiting factor in most cases of such spoilage. The factor of temperature must not be overlooked. The moisture content limit may be materially increased during the winter without evidence of the activity of microorganisms. The agents of spoilage in all the cases under review were primarily molds. The results already given in this paper harmonize in general with those

of McHargue. It has been possible, however, to go farther and indicate more clearly the groups of organisms regularly present and to record the conditions under which certain of them become active factors in spoilage.

Routine mass or dilution cultures show that certain molds are recoverable from practically all samples of meal. Among these are *Rhizopus nigricans* Ehrenberg and some of the mucors which frequently overgrow plate cultures within two days of incubation, although they probably are present only in spore form in the meal. Syncephalastrum, belonging to the same group, is not uncommon. *Aspergillus flavus* and *A. niger* are only occasionally visible factors in the infection of the unground grains, but they always appear as rapidly growing colonies in the mass or dilution cultures made. The brown masses of *A. tamari* Kita are commonly found with *A. flavus*. *A. fumigatus* Fres. and *A. terreus* Thom are frequently present but are quickly overgrown by the more active species already mentioned. *A. repens*, though practically always present, can be found only by careful search in the presence of these rapidly growing forms.

Several strains of Penicillium are found in meal cultures. Penicillia of the group with submerged orange mycelia and of the Citromyces group are probably most common. *Penicillium expansum* Link is reported by McHargue. *P. oxalicum* Thom and Currie is found in many samples of meal, but rarely in miscellaneous cultural work. Strains related to *P. luteum* Zukal and *P. purpurogenum* O. Stoll are frequently present but usually indicate soil contamination rather than active growth in the corn or meal. One sample of corn rotted by a member of this series has been examined, but the conditions shown clearly indicated that the product had contained high percentages of moisture at the time the rotting occurred.

Colonies of Fusarium develop from almost every sample of meal. Infections of this group are so abundant that conidia or grains of meal containing living hyphae are rarely absent. Cladosporium and Alternaria are frequently found but represent spore contamination rather than infection. The other organisms observed in culture from time to time appear to represent excessive contaminations with spores due to unfavorable conditions in the handling of the product, or, in certain species, to actual infection of the grain locally by the mold.

The bacteria found in the fresh samples here considered were predominantly *Bacterium aerogenes*. Certain other organisms have been regularly obtained in culture. When the necessary moisture is present, souring is so characteristic of the product that Round and Gore (7) found the addition of 3 per cent of fresh meal an adequate starter to insure the dominance of lactic acid fermentation in potato silage. Lacto-bacilli were present in four of the nine lots reported in Table 1. According to unpublished records in the Microbiological Laboratory, Round found organisms of this group abundant also in fresh meal, but occasionally

absent in old meal or meal made from old and thoroughly dried corn. Micrococci are constantly encountered in culture but have not been typed. Aerobic spore formers of the mesentericus group are always present, and in spore form they constitute the larger part of the living bacteria in some meals after long storage.

This was clearly demonstrated by a series of experiments upon the possibility of producing a sterile meal with steam, dry heat, or both (unpublished cultural results of Ruth B. Edmondson). The spores of this group survived more heating than could be applied under practical working conditions to the product. Aside, however, from meal so wet as to be unmarketable, these experiments show no evidence of bacterial activity. One sample of apparently sound yellow meal showed the presence of *Bacillus niger* Migula in such extensive numbers that masses of meal placed upon culture media were promptly overgrown and with the agar turned bluish black with this species. The meal was contributed by Dr. S. S. Adams, of Washington, D. C., who reported the feces of a child apparently well to have been blue when fed this meal.

When, however, corn or meal is bottled and incubated at laboratory temperature (20° to 30° C.), those species capable of developing under the conditions presented show active growth. In the authors' series such growth was not detected by physical appearance in products carrying less than 13 per cent of moisture. Certain stone-ground samples of Winton's series (8) showed some evidence of mold activity below that figure. Measurable changes in quality certainly occur in such meals during storage. Some experimental results have suggested the possibility that these changes in such meal are due to the distribution of infected material throughout the mass by the grinding of infected corn. This conflicts with the current trade belief that the natural enzyms of the germinal area are the chief causes of such deterioration, but reflects the findings of Hoffer (5) and his coworkers that even selected seed corn may be extensively infected. Examinations of commercial samples in the Microbiological Laboratory have shown extensive development of molds within the grain itself in corn of other than the higher grades.

In samples carrying 14 to 15 per cent of water the formation of balls and concretions in the meal begins to be evident. The principal agent in their formation appears to be *Aspergillus repens*, although many difficulties are encountered in fixing a minimum moisture percentage for the activity of this species. Changes involving the development of mold mycelium in the meal begin within the limit of 13 to 15 per cent of moisture. Incubation at 20° to 30° C. merely accelerates changes which would progress more slowly in colder places. Moist chamber experiments with meal inside this range of water content show the presence of active mycelia of more than a single species, but principally *Aspergillus repens*. When the percentage of moisture reaches 16, several species are clearly able to grow. Special studies with *Aspergillus*

*flavus* show that very little development of this species occurs below 16 per cent, but that from 16 per cent upward development of this species rapidly increases and the number of forms capable of growing rapidly rises. Among the characteristic saprophytic molds observed under these conditions, in about the order of their abundance under the conditions, are *Aspergillus repens*, *Aspergillus flavus*, *Actinomyces* sp., *Penicillium* sp. and *Citromyces* sp., *Fusarium* sp., *Aspergillus candidus*, *Aspergillus ochraceous* Wilhelm, *Aspergillus tamari*, and *Aspergillus niger*.

Bacterial activity appears to be a concomitant of the disintegration due to mold action in such rotting processes as this. As indicated by Bailey and Thom (*2, Table I*), active disintegration by molds is accompanied by an increase in the water percentage of the sample. Bacteria follow rather than initiate the process in the samples studied, thus becoming a small factor in the merchantable product.

Throughout this investigation a close correspondence has been observed between the flora of deterioration in unground corn and the flora of the milled product.

## SUMMARY

In seeking possible causes for the well-recognized instability of corn meal, cultures show considerable numbers of molds and bacteria to be generally present. Among these the following species of molds were characteristic of many series of cultures: *Fusarium* sp., *Aspergillus repens*, *A. flavus*, *A. tamari*, *A. niger*, *Citromyces* (or *Penicillium* section *Citromyces*) sp., *Penicillium oxalicum*, *P. luteum* varieties, *Mucor* sp., *Rhizopus nigricans*, and *Syncephalastrum* sp., together with various yeasts and yeast-like fungi. Among bacterial groups, the colon-aerogenes group and lacto-bacilli were most abundant in fresh meal. Aerobic spore formers and micrococci were always present and persisted in the stored product.

Within the range of composition found in merchantable meals, no bacterial activity was detected. Only one grade of unbolted meal showed signs of mold development below 13 per cent of moisture. Above 13 per cent moisture, *Aspergillus repens* begins to be an active agent of spoilage somewhere between 13 and 15 per cent of moisture, varying with the form of milling practiced. Several other species of molds are active in meal containing 16 per cent moisture; and numerous forms, including some bacteria, develop when 18 to 20 per cent of moisture is found.

Many samples of corn are found to carry extensive infections with Fusarium, Diplodia, *Aspergillus repens*, or Penicillium, especially in the germinal area and in the tip of the kernel. These sections of the kernel are removed in varying degrees by different milling systems. The bolted meals examined show a corresponding reduction in count of viable organisms as shown by culture.

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# HOPKINS HOST-SELECTION PRINCIPLE AS RELATED TO CERTAIN CERAMBYCID BEETLES

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## INTRODUCTION

In connection with the reported dying of lodgepole pine (*Pinus contorta* Loud.) over extensive areas in northeastern Oregon caused by the mountain pine beetle (*Dendroctonus monticolae* Hopk.) and the threatened invasion by this beetle of the adjacent areas of yellow pine (*Pinus ponderosa* Laws.), detailed investigations were made by the Bureau of Entomology under the direction of Dr. A. D. Hopkins. Manuscript reports of these investigations, submitted in the summer of 1910, showed that the infestation by the beetle in the lodgepole pine was so extensive that there was no hope of controlling it, but that the comparatively small amount of infestation in the valuable stands of yellow pine was such as to warrant the undertaking of control, provided the beetle did not migrate from the lodgepole pine to the yellow pine.

In a letter from Dr. A. D. Hopkins under date of July 30, 1910, relating to a manuscript report of Mr. H. E. Burke, the following statement occurs which appears to be the first written reference to the host-selection principle:

> The more I consider the various features of the problem, the more I am convinced that it is entirely practicable to protect the yellow pine, even if we leave all but the immediately adjacent lodgepole pine to take care of itself. This is based on my belief that the majority of the broods of the beetles which have been breeding in the lodgepole will continue to confine their attack to that species, and gradually diminish with the reduced supply and their increased struggle to adapt themselves to the yellow pine. I may be wrong in this, but it is a matter worthy of careful consideration. Remember, that in all these years, there has been no marked or general migration of beetles from lodgepole to the yellow pine. Therefore, it appears that the broods which are most dangerous to the yellow pine are those which have been breeding in it, and that these are the broods we will have to deal with mainly in our efforts to protect the best bodies of yellow pine.

The control operations that were carried on during the following year, 1911, were confined mainly to the yellow pine area. In manuscript reports by Messrs. W. D. Edmonston and George Hofer on a special examination of the yellow pine and lodgepole pine areas in the summer and fall of 1913, it is stated by Edmonston:

> In 1912 the examination of the areas on which insect control work was carried on during April, May, and June, 1911, showed an average reduction of the infestation on the entire area, 76,430 acres, of close to 85 per cent.

Examinations made this season, 1913, show a still greater reduction in the infestation, in fact, the infestation is so light that it is actually less on the treated areas than it is throughout any other area on this Forest.

and—

There was no reoccupation of the treated areas by broods from the lodgepole infested trees at higher elevations

and by Hofer—

As we reached the summit near the North Powder Peaks we attained an altitude of 8,000 feet; the elevation at the Sheep Ranch is about 4,000 feet. From the summit of this divide for a distance of 10 miles north, 10 miles east, and 16 miles west we noted heavy infestation, both old deadings and the new work also, in both the lodgepole pine and white bark pine, especially on both slopes of Antone Creek.

No new infestation was found on the treated areas on Anthony Creek, Camp area.

This seemed to furnish substantial evidence that the principle would hold.

The principle as defined by Dr. Hopkins [1] is that an insect—

species which breeds in two or more hosts will prefer to continue to breed in the host to which it has become adapted

In order to secure further evidence relating to this principle, the writer, after consulting with Dr. Hopkins, began a series of experiments in 1914 with insects which infest two or more species of wood. The wood-boring Cerambycidae, or long-horned beetles, offered material which was very well adapted to the conduct of such experiments. Many species were easily available which exhibit great diversity in their selection of hosts in nature, as illustrated by those breeding exclusively in a single species of plant and those apparently attacking almost any wood. This variation in host habits at once brought up the following questions: Will those species confined to a single host live in any other, and do the individuals coming from a certain plant of those species breeding in a variety of hosts select the same species of plant on which to oviposit? Again, if such is the case, how do these host strains originate in nature?

As these experiments progressed new problems came up demanding a broadening of the experiments from year to year until, during the season of 1918, over 100 individual experiments were in progress. Fourteen species of insects and 21 species of plants were used, combining to form 45 host strains. It was thought desirable to conduct experiments on more species rather than more intensive experiments on a few species. It will be noted that certain experiments were not carried as far as others, due to the fact that time was not available or due to the absence of the writer at the critical time. At present several points remain to be conclusively settled, and investigation of these will be continued another year or so. Nevertheless it is believed that sufficient data have been accumulated to show definitely the extent to which the influence of the host applies to these insects.

[1] HOPKINS, A. D. ECONOMIC INVESTIGATIONS OF THE SCOLYTID BARK AND TIMBER BEETLES OF NORTH AMERICA. In U. S. Dept. Agr. Program of Work, 1917, p. 353. 1916.

## HISTORICAL

Very few references to the adaptation of insects to their host plants or the variation in their selection of host plants can be found. The most important paper dealing with the subject is that by Pictet.[1] This author shows by several examples, (*Ocneria*) *Porthetria dispar* for one, that caterpillars of the second and third generations may be made to change their preferred food plants and that the adults reared from them exhibit changes in size and coloration. This paper is reviewed, and supplemented with reports of corresponding observations, by Schröder,[2] who in a previous article [3] showed that even nidification (in *Gracilaria stigmatella* F.) and habits of feeding, combined with changes in reproduction (in the beetle *Phratora vitellinae* L.), can be changed and that these acquired characters are transmitted spontaneously from the third generation.

In 1907 and 1908 Paul Marchal [4] succeeded in transferring numerous specimens of *Lecanium corni* Bouché from the peach (*Amygdalus persica* Linn.) to the black locust (*Robinia pseudacacia* Linn.). Eggs hatched and larvæ developed on the new host plant, spreading out over the leaves in large numbers, and in the fall migrating from the leaves to the wood for hibernation. In the summer of 1908 the insects completed their development and had then the large size, deep coloration, and characteristic appearance of the insect described by Douglas as *L. robiniarum*, the attacks of which on the black locust had been severe in several European localities. This indicated that *L. robiniarum* was only a race of *L. corni*, resulting from individuals that had become transferred in some manner from the peach to the introduced American black locust. Dr. Marchal found great difficulty in reestablishing on the peach individuals of *L. corni* produced on the black locust.

There are other records showing the acquired adaptation of certain species to new host plants, similar to those here cited. The practical application of such phenomena, however, has, so far as can be ascertained, first been recognized by Dr. A. D. Hopkins (referred to on p. 189 of this article) and presented by him in concrete form.

In a paper prepared by M. Joseph Capus [5] on a nematode disease of peas in the Gironde and read by Paul Marchal at the session of July 10, 1918, of the French Academy of Agriculture there is a record of injury to peas by a fungus (*Fusarium vasinfectum* var. *pisi* van Hall, considered as the conidial form of *Necosmopora vasinfecta* E. F. Smith, accompanied by

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[1] Pictet, Arnold. INFLUENCE DE L'ALIMENTATION ET DE L'HUMIDITÉ SUR LA VARIATION DES PAPILLONS. *In* Mém. Soc. Phys. et Hist. Nat. Genève, v. 35, fasc. 1, p. 45–127, pl. 2–5. 1905.

[2] Schröder, Chr. DIE LITERATUR ÜBER DIE FÄRBUNG DER INSEKTEN DES JAHRES 1905. *In* Ztschr. Wiss Insektenbiol., Bd. 3, p. 162–164. 1907.

[3] ——— ÜBER EXPERIMENTALL ERZIELTE INSTINKTVARIATIONEN. *In* Verhandl. Deut. Zool. Gesell, Jahresversamml. 13, p. 158–166. 1903.

[4] Marchal, Paul. LE LECANIUM DU ROBINIA. Compt. Rend. Soc. Biol. [Paris], t. 65, p. 2–5. 1908.

[5] Capus, Joseph, and Marchal, Paul. SUR LA MALADIE VERMICULAIRE DES POIS DANS LA GIRONDE. *In* Compt. Rend. Acad. Agr. France, t. 4, no. 25, p. 712–716. 1918.

the nematode *Heterodera schachti* Schmidt). After pointing out the interdependence and relation of the two, M. Capus says:

One might ask himself why this species, everywhere known for its injury to beets, does not establish itself on this plant in the Gironde and appears so abundantly on peas.

Following M Capus's explanations of this phenomenon, Dr. Marchal observed.

Among the very interesting facts pointed out by M. Capus in his note I wish to call attention to the following: That injury to beets by *Heterodera* in the Gironde is not constant is rare. We should recall in this connection the observations, already old but interesting, of the Dutch naturalist Ritzema-Bos. He has shown that when nematodes multiply in course of years without interruption on the same host, biologic races are formed adapted to this host which later pass to other vegetation with greatest difficulty, even when these are of those preferred by the species. It must be, therefore, that, by virtue of the conditions of pea culture in the Gironde, a race of *Heterodera schachti* was formed especially adapted to peas and to the attack of which beets are resistant up to a certain point. There is no doubt that it will adapt itself to beets cultivated for a number of years in succession in the same soil infested with *H. schachti.*

The experiments conducted at the Gipsy Moth Laboratory[1] show that of the many plants tested a decided variation was found in regard to the susceptibility to attack by this insect. The plants were divided into four groups: I, favored species; II, favored food species after early stages; III, species on which a small proportion may develop; IV, species that are unfavored food. These results show that, although this insect has a wide variety of hosts on which it is capable of feeding, certain ones are selected in preference to others in the natural forests. As far as known, no observations have been reported showing whether or not several years' feeding on any particular host produces a strain which selects that in preference to others.

In a recent paper Dr. C. T. Brues[2] writes as follows (on p. 328–329):

It has been claimed that the food habits may be modified experimentally, in that caterpillars reared on a strange plant (where they could be induced to select it) give rise to moths whose progeny more readily accept the new plant. It is very difficult to accept such evidence, at least as having any general application, without very clear and incontrovertible proof. If such transformations can occur so easily and become hereditary so quickly they should have entirely destroyed the coherent habits now existent, during the enormous period which has elapsed, for example, since the violet-feeding Argynnids were differentiated, since the holarctic and nearctic Vanessids have been separated, or while the world-wide Aristolochia-feeding Papilios were attaining their present distribution. That such a change has actually occurred in the case of other groups seems equally evident, although, as has been shown, we can more easily believe that they may have arisen through mutations in maternal instinct not incompatible with larval tastes and then only in extremely rare cases and confined to certain groups.

## METHODS OF CONDUCTING EXPERIMENTS

In connection with the experiments by the writer several types of cages, the particular type determined by the amount of material handled

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[1] MOSHER, F. H. FOOD PLANTS OF THE GIPSY MOTH IN AMERICA. U. S. Dept. Agr. Bul. 250, 39 p., 6 pl. 1915.

[2] BRUES, Charles T. THE SELECTION OF FOOD-PLANTS BY INSECTS, WITH SPECIAL REFERENCE TO LEPIDOPTEROUS LARVAE. *In* Amer. Nat., v. 54, no. 633, p. 313–332. 1920.

and the exact conditions required, have been used in confining the colonies of beetles. It is essential to duplicate as closely as possible the conditions in which the insects are found in nature.

For the larger logs and for experiments in which a large amount of material was used, an open wire insectary was constructed. This insectary is 40 feet long by 10 feet wide by 7 feet high. The foundation is of concrete, the side walls and top of 18-mesh galvanized wire screening, and over all a removable lattice-work roof was placed. This roof was adjusted to simulate shade conditions in the woods. It was removed in winter and replaced in summer. The floors were made of ashes to give good drainage. Cross partitions divided the insectary into seven compartments of different sizes. One room was entirely boarded in and roofed over. It was used for seasoning wood. Another was lined with cheesecloth, which was used for holding different cuts of wood under natural conditions until desired for use. In the other compartments were placed logs containing various species of insects. Where no danger is present of any infestation from the original host wood into cuts of different wood, it was possible to place several beetle species in the same compartment and continue their breeding in the same host from year to year. In this way forms such as Callidium in pine (*Pinus* spp.), *Neoclytus capraea* Say in ash (*Fraxinus* spp.), and *Cyllene pictus* Drury in hickory (*Hicoria* spp.) were placed together.

The smaller insects, especially those in twigs and branches, were confined in glass museum cylinders of various sizes. The tops were kept in place so that a very constant degree of humidity could be maintained. This cage was found to give best results for the development of the larvæ and, as no sand was needed, the adults were easily found in the cages. These jars were kept under a roof all the year in another insectary.

Each of these insects has a particular preference for a certain condition or seasonal cut of wood. Also in some species the adults require food before ovipositing, consisting of green bark from twigs, leaves, or fungus spores. In the latter case the pustule of the chestnut blight (*Endothea parasitica* (Murr.) P. J. and H. W. Anderson) was used. Again, some require much moisture, others rather dry surroundings. The determination of these factors sometimes delayed the successful continuance of a species for a year or more. When a new colony was collected from nature it was ascertained as nearly as possible when the tree died and the condition of the wood, also what degree of humidity was desirable. For instance, those insects naturally feeding in dead branches of a standing tree required drier conditions than those attacking branches fallen to the ground.

In order to meet these conditions, wood of the various species used was cut every month or every other month of the year and stored under different conditions. Part was placed in the dry shed for dry seasoning, part hung or stood up in the open-air cage for normal air seasoning, and

part laid on the ground in the wire cages for wet seasoning. The condition of the wood on which the insects were first found eliminated the use of certain of these periodic cuts and conditions of seasoning. However, the first-year adults were usually caged with the choice of many of these cuts and the one infested most heavily was considered as the optimum condition and used afterwards for continuation of the successive broods. The optimum cut could only be determined when sufficient material was given for the number of insects present, as an unfavorable cut may be attacked when the adults are confined on it without sufficient optimum material.

Wood used a month or two after being cut is spoken of as green or freshly cut material.

In many cases wood from several individual trees was used to avoid any possibility of offering an undesirable individual.

To illustrate the variation in optimum conditions of wood, several examples are given: *Callidium antennatum* Newm. requires wood dry-seasoned over winter; *Neoclytus capraea*, wood cut during the late winter with the inner bark still sappy; *Liopus alpha* Say in hickory, twigs cut in the early fall, air-seasoned for a while and then left on the ground over winter so that the inner bark sours somewhat. (This condition is brought about by the girdling habit of *Oncideres cingulata* Say.)

Several terms which may need explanation are used in reference to the species of host wood: *Primary host*, or *original host*, refers to the wood in which the insect is found in nature and first caged in these experiments; as *secondary host* is understood wood in which a colony has been successfully produced in the experiment, but it may or may not be recorded as a host in nature; an *unfavorable host* is one not recorded from nature and in which attempts to produce a colony have not been entirely successful.

All experiments conducted are here given, although a few have been unsuccessful or have given no results. Occasionally failure to continue a colony is recorded. In all cases an explanation can not be given. It may be because of an improper cut of wood or of a peculiarity of the individual host. In one case partial failure was due to a nematode parasite causing sterility of the females; in another, the parent insects were entangled in spider webs and killed before ovipositing.

Reference is made to larval transfers from one host to another. This is accomplished by making a smooth cell through the bark of the new host, partially filling it with frass from the larval mines of the original host, then placing the larva in this cell and finally tightly fixing a piece of bark over the cell. Such transfers do not injure the larva or affect its development. Many cases of transfer to the same host resulted in the survival of every larva.

These experiments were conducted at the Eastern Field Station, East Falls Church, Va., and all flight dates of the adults and times of cutting of the wood refer to this locality unless otherwise stated.

## OUTLINE OF EXPERIMENTS ON EACH SPECIES

### XYLOTRECHUS COLONUS. EXPERIMENT I

*Xylotrechus colonus* Fab. is found in nature in a wide variety of hosts. In fact, it feeds in nearly all hardwood deciduous trees of the eastern and central United States. It shows little or no preference for any exact condition of the wood, except that it will not attack perfectly seasoned material. The larvæ can be found in dying standing trees or in logs felled in any month of the year provided they still contain a certain amount of moisture.

The first flight of the year occurs in the last week of May or first week of June, reaching the maximum in about two weeks. A few adults emerge sporadically throughout the summer. From eggs deposited in June a few adults usually emerge in September, but the main brood remains as larvæ until the next spring. These fall adults have never oviposited under confinement.

The larvæ feed entirely beneath the bark, or in the bark if it is thick. The pupal cell is made in the outer sapwood or in the bark.

The wood of all species for this experiment was cut on April 15 unless otherwise stated. The colony was started by felling a red oak tree in March, 1914. The wood was attacked during that June, caged soon afterwards, and the colony has since been maintained in red oak. From the original oak form colonies were secured in hickory (Hicoria), chestnut (*Castanea dentata* (Marsh.) Borkh.), locust (*Robinia pseudacacia* Linn.), red maple (*Acer rubrum* Linn.), and ash (*Fraxinus* sp.), in the following manner:

QUERCUS. EXPERIMENT I.—During May, 1915, hickory logs were placed in the cage with oak intended to carry on the colony. Many adults were present, somewhat over 100, and the hickory as well as the oak was subsequently found infested.

In June, 1916, in the same cage stocked with oak for continuing the colony, chestnut and hickory wood was placed. There was again an overabundance of adults and all woods were infested.

In June, 1917, oak was placed in this cage to continue the colony and also ash, chestnut, locust, hickory, and red maple logs, all cut in February except the hickory, which was cut in April, 1917; extra pieces of chestnut and maple, cut in November, 1916, and September, 1916, respectively, were also placed in the cage. There was an abundance of adults. In July these logs were examined and it was found that the oak was heavily infested; the chestnut and hickory were lightly infested; the ash, maple, and locust had no infestation. This same year, 1917, adults were isolated on ash ($I^5$), maple ($I^4$), and locust ($I^3$) with results as described in later paragraphs.

In June, 1918, two pairs of adults from oak were caged on a collectior of oak, hickory, ash, maple, and chestnut logs, all cut April 15, and o about equal size. Examination in July showed that the oak was heavily infested (over 50 larvæ present), the chestnut contained 10 larvæ, th hickory 7 larvæ, and the ash and the maple none.

At the same time a similar cage was prepared, and six pairs of adult were placed in it to test the influence of a greater number of beetle on the selection of hosts. The results showed the same relative propor tion of infestation except that ash also was attacked. The maple wa not infested.

In June, 1919, this experiment was repeated with the same condition except that the hickory sticks were accidentally omitted. They wer examined in July and the infestation was as follows: The woods in the cag of two pairs of adults contained 28 larvæ in oak, 22 in chestnut, and non in ash and maple; that of six pairs contained over 50 in oak, 19 in chest nut, and none in ash or maple.

HICORIA. EXPERIMENT I[1].—May, 1915, hickory logs were placed (a before described) in the oak cage with the wood intended to carry or the colony. They were infested and in subsequent years kept isolatec and continued as the hickory form.

In June, 1916, oak was placed in this cage, together with the hickory to continue the colony, and was subsequently found heavily infested.

In June, 1917, together with the hickory for reinfestation, chestnut and locust were placed in the cage. An examination in July showed that the chestnut contained a few larvæ and the locust none.

In June, 1918, two pairs of adults were isolated in a cage containing oak, hickory, ash, chestnut, and maple, all cut April 15, and of equa size. The results showed that the hickory was heavily infested by over 50 larvæ, the oak contained 7 larvæ, the chestnut 1 larva, and the maple and ash none.

In June, 1919, selection tests and selection quantity tests were carried out with this strain. The quantities and cuts of wood were the same as before, except that oak was accidentally omitted. In one cage two pairs of adults were isolated, the resulting infestation being hickory 18 larvæ, chestnut 12 larvæ, maple and ash none. In another cage six pairs of adults were isolated, the resulting infestation being hickory over 50, chestnut 40, and maple and ash none.

CASTANEA. EXPERIMENT I[2].—In May, 1916, chestnut logs were placed (as before described) in the oak cage with the wood intended to carry on the colony. The wood was heavily infested, and these individuals have since been confined to chestnut.

In May, 1917, together with the chestnut, hickory was placed in thi cage. The hickory was lightly infested.

In June, 1918, two pairs of adults were isolated in a cage containing oak, hickory, ash, chestnut, and maple, all cut April 15 and of equa

size. An examination in July showed that the oak and chestnut were equally well infested, the hickory contained one larva, and the maple and ash none.

Robinia. Experiment $I^3$.—In June, 1917, eight adults from oak were isolated on black locust cut in February, 1917. The adults laid eggs, but all died later.

In June, 1918, the experiment was repeated with 15 adults and wood cut April 15. Many young larvæ entered the bark, but by August 15 nearly all had died, and none lived to transform the next spring. The experiment was not repeated in 1919.

Acer rubrum. Experiment $I^4$.—In June, 1917, eight adults from oak were isolated on a piece of red maple cut February 1, 1917. A few larvæ lived and three adults (two males and one female) emerged in 1918. They were isolated in a cage containing oak, chestnut, hickory, ash, and maple, all cut April 15, but no infestation occurred in any wood. During August, 1918, twenty larvæ were transferred from oak to maple to continue the species in this host. A few adults emerged in 1919, and were recaged on maple to develop a larger colony, which will be continued several years before testing the selection again.

Fraxinus. Experiment $I^5$.—In June, 1917, eight adults from oak were isolated on a piece of ash cut January 1, 1917. A fair infestation occurred, but the larvæ developed slowly, and in May and June, 1918, only seven adults (both sexes represented) emerged, while many larvæ were still in the wood. These adults were transferred to a cage containing oak, chestnut, hickory, ash, and maple, all cut April 15, but no infestation occurred in any wood. Larvæ were again transferred to ash, and a few adults emerged in 1919. These were recaged on ash, and several adults emerged in June, 1920, but failed to develop any larvæ in the new wood.

I, $I^1$, $I^2$, $I^4$.—During June, 1920, adults emerged from the oak, hickory, chestnut, and maple; strains and adults from all were recaged on the same wood and produced new colonies. No selection tests were made, and these strains will be continued for several years in the same wood before similar experiments are again attempted.

## CONCLUSIONS

The original oak strain of *Xylotrechus colonus* shows a decided preference for a few woods, notably oak, chestnut, and hickory. Two years' trial failed to produce larvæ capable of completing their development in locust, while the ash and maple colonies were maintained with difficulty. In nature these woods (ash, maple, and locust) have been found containing thrifty colonies of this species.

Originally the oak strain showed little preference as between oak, hickory, and chestnut; yet, after several years, strains were developed in each wood that showed a growing preference for the given wood.

The number of insects present under identical conditions influences their selection of hosts. When few are present they concentrate on original or favored hosts; when more than can successfully oviposit on original hosts are present, less favored hosts are taken.

CYLLENE PICTUS, HICKORY HOST STRAIN. EXPERIMENT II

The larvæ of *Cyllene pictus* feed almost exclusively in hickory. A few specimens have been taken in wild grapevine (*Vitis* sp.), mulberry (*Morus rubra* Linn.), osage orange (*Toxylon pomiferum* Raf.), and hackberry (*Celtis occidentalis* Linn.), but such instances are rare and of very local occurrence. In one locality near Harrisburg, Pa., all except one of these unusual food plants have been recorded. This borer is found generally distributed east of the Mississippi River. The optimum condition of wood is that cut during the winter, preferably in January, and left lying on the ground. November cuts are sometimes attacked, but no wood is suitable unless the inner bark is still sappy. Sticks cut at the time of emergence are too green for attack.

The first emergence occurs about the middle of April and continues for three weeks. By September the larvæ are full grown and have constructed their pupal cells in the wood. They soon pupate, and in this stage they overwinter. The larvæ feed about equally beneath the bark and in the wood.

These experiments were started in April, 1915, when adults were found ovipositing on a hickory log cut during the winter at Falls Church, Va. The strain has since been continued in January and February cuts of this wood, and other host strains have been attempted with varying success in grape (*Vitis* sp.), locust (*Robinia pseudacacia*), ash (*Fraxinus* sp.), and mulberry (*Morus rubra*). Experiments were conducted as follows:

VITIS. EXPERIMENT II[1].—In April, 1917, a piece of grape, cut in January, was placed in the same cage with much hickory used for the continuation of the hickory form. This grape was not infested.

June 26, 1917, sixteen larvæ, about half grown, were transferred from hickory to grape cut in January. Nearly all these larvæ lived, and the following spring 12 adults emerged. They were isolated in a cage containing several pieces of grape and one of hickory, both cut in February. Examination in June showed the grape to be heavily infested while the hickory contained no larvæ.

In April, 1918, a large number of adults emerged from the grape. Two pairs were isolated in a cage containing one piece of grape 2 inches in diameter and 2 feet long, and one piece of hickory of the same size, both cut in January. Examination in July showed the grape to be very heavily infested, whereas the hickory contained only a few larvæ.

ROBINIA. EXPERIMENT II[2].—April 21, 1917, three females and two males from hickory were caged on a piece of locust cut a month previously. The females laid all their eggs on the locust and the young

larvæ bored through the bark, but by the middle of June all had died. June 15, twelve larvæ 5 mm. long were transferred to locust and these all died by July 11, when three more, over half grown, were transferred. These lived to construct pupal cells and pupated, but all the pupae died during the winter.

In April, 1918, five adults (three females and two males) from hickory were caged on locust cut during January, 1918. The females laid all their eggs, but only a few larvæ lived. These constructed pupal cells and pupated beneath the bark. In locust the larval mines are not normal, lying in almost all cases immediately beneath the bark instead of extending deep into the wood. About half the larvæ made pupal cells in the outer sapwood while the others pupated beneath the bark instead of, as normally, deep in the wood.

April 20, 1919, a total of six adults had emerged and two pairs were caged on pieces of locust and hickory cut in January, 1919. An examination July 16 showed no infestation in either.

FRAXINUS. EXPERIMENT II[3].—April 24, 1917, three females and two males were isolated on ash cut during January. The females laid all their eggs and the young larvæ bored through the bark, but all died before June 15. At this time fourteen larvæ 5 to 7 mm. long were transferred to the same ash, and all died within a month. July 15, five more, over half grown, were transferred. They mined extensively beneath tbe bark, but all died before the end of September without pupating.

July 24, 1918, fifteen larvæ, one-half to three-fourths matured, were transferred to ash cut in January.

April 21, 1919, a total of eight adults had emerged. One pair was caged on the January cut of ash, and two pairs were caged on January cuts of ash and hickory.

July 16 the wood was examined, but in no case was it infested.

MORUS. EXPERIMENT II[4].—April 29, 1918, three females and two males from hickory were caged on mulberry cut in January. The females laid eggs, and a very heavy infestation was secured. They developed normally and suffered little more than the normal rate of mortality experienced in hickory.

April 21, 1919, a total of 17 adults had emerged; two pairs were transferred to a cage containing two pieces of mulberry and one piece of hickory cut during January, 1919. In another cage containing the same quantity of wood four pairs of adults were transferred. In neither case was the quantity of mulberry sufficient to permit the development of all the larvæ. Each piece was 2 inches in diameter and 14 inches long.

July 16 the cages were examined, and that containing two pairs of adults was infested as follows: Hickory 6 larvæ, mulberry over 30; that containing four pairs, hickory 13 larvæ, mulberry very heavily infested, over 40.

SEASONED HICORIA. EXPERIMENT II[a].—Attempts were made in April, 1917, to develop a colony adapted to seasoned wood by caging two females and one male on wood cut in October and dry seasoned. Eggs were laid and larvæ entered the bark but developed very slowly, never entering the wood. Only three lived to pupate, and these made their pupal cells between the bark and wood. All three pupæ died during the winter.

In May, 1918, the experiment was repeated with three females and three males and wood cut in November, 1917. In the fall of 1918 a number of larvæ lived and pupated, but all were below normal size. Only a few adults emerged in April, 1919, and these were below normal size.

II[b].—Dr. A. D. Hopkins, in 1916, recorded a dying hickory tree heavily infested by *Cyllene pictus* with no evidence of primary injury from other causes.

This suggested that a strain capable of attacking living trees might be produced, and attempts were made to secure a colony in such a tree. A small hickory 3 inches in diameter was selected and entirely stripped of leaves August 11, 1916. April 30, 1917, it was again defoliated, and 80 adults were caged on it. The adults laid eggs and the young larvæ entered the bark, causing sap to flow from the wounds. However, all died after growing to 3 mm. in length.

In April, 1918, the tree was again defoliated, and 156 adults were caged on it. The same results were observed.

During both years the tree put out healthy foliage after artificial defoliation, but it died in August, 1917. In no case did the Cyllene larvæ live to mine more than ¼ inch beneath the bark.

QUERCUS. EXPERIMENT II[5].—In transferring adults during the spring of 1918 to new hickory wood to continue a large colony, a piece of oak was unintentionally left in the cage. This cage contained six large hickory logs 4 to 6 inches in diameter and 5 feet long. The oak log was 3 inches in diameter and 4 feet long.

During September, 1918, work of Cyllene was noticed on this piece of oak, and in the spring of 1919 it was separately caged. Five adults emerged in April—all very small, much below normal size.

Two females and one male were transferred to a cage containing only oak; one pair to a cage of oak and hickory. These cages were examined July 16. Neither wood of the selection test was infested, but the oak wood on which two pairs were caged contained a few very small larvæ.

II[s1].—To test the influence of host selection on the condition of host. In April, 1918, two males and two females from hickory were isolated in a cage containing a piece of grape and a piece of hickory of equal size—the grape of optimum cut, January, 1918, the hickory less favorable, November, 1917. Examination in July showed the grape to be heavily infested while the hickory contained very few larvæ.

$\mathrm{II}^{32}$.—To test the influence of an overabundance of adults and scarcity of the primary host on the host selection. Three pairs of adults from hickory were caged on a small piece of grape and a small piece of hickory (each 2 inches in diameter and 1 inch long), each cut during January, 1918. Examination in July showed both woods to be infested, the grape containing a few more larvæ than the hickory.

In 1920 no adults emerged.

II, $\mathrm{II}^{4}$.—In 1920 only two strains were continued, those in hickory and those in mulberry. No attempt was made to reestablish the others that failed.

CONCLUSIONS

This species, although most commonly found in hickory, will readily adapt itself to several other plants, notably mulberry and grape, both of which are recorded as natural hosts.

In some unfavorable hosts, or in an optimum host in an unfavorable condition, the larvæ may become established, but the mortality is high and the progeny seem to be sterile.

After one year's feeding in a new host the larvæ may select that host in preference to others.

The selection of a host is influenced by the number of adults present and the quantity of the primary host, in that adults will prefer a secondary host to overinfesting the original host.

The selection of a host is influenced by the condition of the host, a favorable condition of secondary host being preferred to an unfavorable condition of the original host.

The optimum condition of any host capable of properly supporting growth of the larvæ is of very restricted limits.

CYLLENE PICTUS, GRAPE HOST STRAIN. EXPERIMENT III

This is the same species as previously discussed, having the same biological habits except that this host strain in grape (Vitis) was taken in nature at Hummelstown, Pa., in January, 1916, by J. N. Knull. Since then the colony has been continued at East Falls Church, Va., in grape cut in January or February. Other host strains have been produced and experiments conducted as follows:

HICORIA. EXPERIMENT $\mathrm{III}^{1}$.—When these adults emerged from grape (May, 1916), three females and two males were isolated in a cage containing 10 pieces of grape and 1 piece of hickory cut in February, 1916. No eggs were laid on the hickory. April 17, 1917, two pairs of adults were isolated on hickory cut in January, 1917. Eggs were laid and the larvæ developed but not so rapidly as in the grape. June 15 they were under normal size. By September only two larvæ were alive. One of these pupated but died during the winter.

Many adults emerged from the grape in 1918 and five pairs were caged on three small pieces of grape 1½ inches in diameter and 2 feet long and one piece of hickory about the same size, both cut in January, 1917. In July they were examined, and both hickory and grape were heavily infested.

QUANTITY SELECTION. EXPERIMENT III[a].—To again test out the effects of host selection when an insufficient amount of wood is given than that required for the number of adults present, in April, 1918, two pairs from grape were caged on grape and hickory cut in January, 1918. The piece of grape was 2 inches in diameter and 1 foot long, the hickory 2 inches in diameter and 2 feet long. An examination in July showed both grape and hickory infested.

This experiment was repeated in April, 1919, using two pieces of grape and one piece of hickory, all of optimum cut and equal size. In one case 1 pair of adults was isolated, in another case 3 pairs were used. The wood on which 1 pair was caged contained 5 larvæ in hickory and 3 larvæ in grape; that on which three pairs were caged contained 4 larvæ in hickory and over 25 in grape.

The grape colony was not continued in 1920.

CONCLUSIONS

This host variety from nature had acquired a decided preference for grape.

The selection of a host is influenced by the quantity of wood present for a given number of adults, in that the adults will select a new host in preference to overinfesting the original host.

The tendency in this species in nature to confine itself to a certain host, either hickory or grape, is not as marked as in some other species.

CYLLENE PICTUS, HICKORY STRAIN II × GRAPE STRAIN III

In order to determine whether crossing of these two host strains would influence the progeny in the selection of the host, males and females were isolated from their pupal cells in the spring of 1917. April 17 three females from hickory and two males from grape were isolated in a cage containing hickory. Two females from grape and two males from hickory were isolated on grape. In neither case did mating occur as readily as when both sexes from the same host were paired. The sexes often approached each other and moved away before finally copulating.

Good infestations were secured in both cases. In April, 1918, one pair from hickory was caged on equal amounts of grape and hickory cut in January, 1918. Only the hickory was infested. Two females and two males from grape were isolated on the same amount of grape and hickory cut in January, 1918. Both woods were infested. These pieces were all 2 inches in diameter and 18 inches long.

CONCLUSIONS

This crossing of the two host forms had no influence on the selection of ...sts.

The amount of wood and number of adults present influence the host ...lection, as shown when one female and two females were given the same ...nount of wood.

CYLLENE CRINICORNIS. EXPERIMENT XLI

*Cyllene crinicornis* Chev., found in the southwestern United States, is ...own to feed only in mesquite (*Prosopis juliflora* (Swartz) de C.) and ...casionally on an allied legume, paloverde (*Parkinsonia microphylla* ...orr.). In general its biology is similar to that of *C. pictus*, and it pre...rs the same conditions of wood. Adults begin emerging in the natu...l range during late February, and part of this generation emerges in ...ptember.

Mesquite infested with these larvæ was sent to Falls Church, Va., by ... E. Snyder from San Antonio, Tex., April 27, 1917. Adults emerged ... Falls Church the following May and were caged on mesquite cut in ...arch, 1918. A good infestation was secured and has since been con...nued on mesquite.

ROBINIA. EXPERIMENT XLI[1].—In May, 1918, two pairs were isolated ... locust cut February, 1918. May 31 the females were dead, and the abdomens were dissected and found to contain eggs. Probably no eggs were laid, and in July no evidence of larval work could be found.

July 24, 1918, seven nearly matured larvæ were transferred from the mesquite to locust cut January, 1918. During May, 1919, five adults emerged. One pair was caged on locust cut in January, 1919, and one pair on both locust and mesquite. The mesquite was cut October, 1918.

July 16, 1919, these cages were examined, and the selection test showed that mesquite was not infested, whereas the locust contained several small larvæ. The cage containing only locust was lightly infested. None of these larvæ transformed in 1920.

CONCLUSIONS

The locust was such an unfavorable host that the adults would not oviposit on it, but larvæ may live and transform for one or two genera...ions when forced to take it.

CALLIDIUM ANTENNATUM. EXPERIMENT IV

Some confusion exists as to the taxonomy of the blue species of Calli...lium allied to *Callidium antennatum*. A number of species have been ...lescribed of questionable validity. Two species have been experi...nented with—*C. antennatum* and *C. janthinum* Lec. These two adults ...re easily separable, and their habits are also quite distinct. The

former, so far as the Forest Insect records are concerned, feeds only pine (Pinus) and spruce (Picea), and for this discussion will be confine to the form occurring in the northeastern United States. It shows decided preference for a certain condition of the host, requiring woc that has been cut in the early fall or winter and is well seasoned. Whe the inner bark is still sappy the insects will not normally make the attack.

Adults first appear about the middle of April, and the flight peric continues about a month. One year is required to complete the li cycle. The larvæ feed beneath the bark until half grown, then ent the wood to construct a long pupal excavation, at the end of which tl pupal cell is chambered off.

These experiments were started in December, 1916, when infest Virginia pine was caged. In 1916 and the following years the colo was continued in seasoned pine, and a form was also developed in spru and freshly cut or green pine. Unsuccessful attempts were made produce a juniper (Juniperus) strain. This insect has been reported feeding in juniper (Juniperus) and maple (Acer).

JUNIPERUS. EXPERIMENT IV[1].—In April, 1916, juniper was plac in the cage together with pine; in addition, about 20 adults were isolat on a stick of juniper (both woods were cut in January, 1916). In neitl case was the juniper attacked, and the females isolated on juniper fail to oviposit.

The same test was repeated in 1917 with juniper cut in October, a the same results were obtained. During June and July, 20 larvæ, frc small to over half grown, were transferred to juniper. All finally die some living a month. None increased in size before death.

PICEA. EXPERIMENT IV[2].—April 12, 1916, a piece of season spruce was placed in the cage, together with the pine, for reinfestatic An examination in June showed only one larva in the stick, and this w far below the normal size of those in pine. By July 11 this larva h died. It is possible that more eggs were laid on the spruce but the lar died earlier.

June 29, 1916, nine larvæ about half grown were transferred to t same spruce wood. July 11, 1916, three larvæ were alive and 17 mc were transferred. From these larval transfers 10 adults were secur in April, 1917. They were caged on four small pieces of seasoned spru and a piece of seasoned pine placed in the cage for one week, both cut October, 1916. Examination in July showed the spruce well infest but only 4 larvæ were found in the piece of pine.

In 1918 the same experiment was repeated, four females and thi males from spruce being caged on four sticks of spruce and one of pi cut in September, 1917. An examination in July showed that the spru contained many larvæ, but none were found in the pine.

In 1919 this was again repeated, but the results showed that pine was infested while the spruce contained no larvæ. Both woods were of November cut, but it is not likely that this would have such a decided influence. However, six adults were isolated on spruce to continue the colony, and this wood was very lightly infested. No explanation can be offered as to the reason for this discrepancy from former results unless the spruce wood was in an unfavorable condition.

During the same season another cage was prepared of pine cut during November, 1918, and green spruce cut in April, 1919. The pine was heavily infested; the spruce contained no larvæ.

EXPERIMENT IV². To test effects of condition of wood on selection.—April 23, 1920, one pair of adults from spruce was caged on a favorable cut of pine (November) and freshly cut spruce (April). When the woods were examined on July 5, 1920, the pine contained many larvæ while the spruce contained none. At the same time a pair was caged on November pine and November spruce, both optimum cuts. Several days later the female was found dead in the cage and had laid no eggs.

ACER. EXPERIMENT IV³.—As maple has been reported as a host of this species, attempts were made in 1916 to start a colony in this wood. On July 6, six half-grown larvæ were transferred to a seasoned piece of wood, but by July 21 all but one had died and it was smaller than when transferred. This one died soon after. None of the larvæ fed on the maple.

GREEN PINE. EXPERIMENT IV⁸.—In 1916 some of the wood used to carry this colony along was cut March 1, and consequently little seasoned. It was very unfavorable for the ovipositing of the adults, but some eggs were laid on the pieces. The larvæ developed slowly and at the time of pupation were below normal size. The adults secured in 1917 averaged about one-half normal size. They were caged again on wood cut in March, 1917. Adults were secured in 1918 and again caged on the same condition of wood, and a good infestation was secured. While the author was absent for a month from the field station in the summer of 1918 these larvæ were all killed by a fungus. The sticks were on the ground and so were caught in a period of rainy weather and were water soaked.

EXPERIMENT IV, IV².—In 1920 the pine and spruce strains were continued in the same wood.

### CONCLUSIONS

The pine form shows a decided preference for that host.

It can live in spruce and then shows a decided preference for that host.

It will not live in juniper or maple.

In producing a new host strain a high mortality occurs in the young larvæ.

A colony can be produced in a host which is in an unfavorable condition, but the resulting adults are below normal size. Owing to the failure to continue the colony it can not be stated whether or not such a strain would show preference for the new condition of the host by selecting it voluntarily.

CALLIDIUM JANTHINUM. EXPERIMENT V

Under *Callidium antennatum* reference was made to *C. janthinum* Lec. It is distinguishable from the former by its smaller size, shining surface, and bluish green color of the adult, by the fact that the larva feeds only in juniper, and that the adults emerge about four weeks later in the spring. It requires wood which was cut during the late fall and which has not seasoned in contact with the ground. It will oviposit in greener wood than *C. antennatum* although the inner bark should not be sappy.

The first flight occurs during the first to third week in May and continues about two weeks. One year is required to complete the development. The larvæ feed beneath the bark until half grown, then excavate long pupal chambers, the ends of which are plugged off for the transformation cell.

These experiments were started with a lot of infested juniper branches from Hummelstown, Pa., collected by J. N. Knull in April, 1916. May 2, six adults emerged and were caged on juniper cut in April and rapidly dried in the house. Since then the colony has been continued each year in September and November cuts of juniper, which are preferred.

PINUS. EXPERIMENT V[1].—May, 1917, two pairs were isolated on pine cut in November, 1916. A few eggs were laid from which larvæ hatched and entered the bark. By July 10 all had died. The same test was repeated in 1918 with similar results.

CONCLUSIONS

This species shows a decided preference for juniper and will not develop in pine from early stages. Larval transfers to pine with nearly matured larvæ were not made.

CALLIDIUM ANTENNATUM AND C. JANTHINUM. V×IV; IV×V

Because of the taxonomic confusion between these species (cited previously) and with the idea that a crossing of these two forms might possibly influence the selection of a host, attempts were made to cross the species.

V×IV.—In May, 1917, four females of the juniper form were crossed with two males of the pine form and were caged on juniper. Both species had been previously isolated from the pupal cells to avoid all possibility of mating. These insects immediately mated, and the eggs were laid on the juniper from which a good infestation was secured.

April 17, 1918, the first adult emerged from this cross. The second adult emerged April 25. May 3 and 4 eight adults emerged. All the 1918 adults resembled the juniper form in color. These adults were all isolated in a cage containing juniper and pine cut in November, 1917. An examination in July showed only the juniper to be infested, but by a very light brood. During the remainder of the summer all died except three larvæ which constructed pupal cells. May 5, 1919, two males and one female emerged and one pair was caged on juniper cut in November. A light infestation occurred. May 12, 1920, five adults emerged and were recaged on juniper. The selection of pine and juniper was not again tested.

IV×V.—These same species were mated in 1917 by making the reciprocal cross (males from juniper and females from pine) aud caged on pine. The sexes did not mate readily, not noticing one another for some hours after being caged together. However, several matings finally took place and eggs were laid on the pine. Young larvæ developed but all died later. The same experiment was repeated in 1918 with the same results. Conditions were similar to those in the previous experiment.

## CONCLUSIONS

The crossing did not influence the selection of a host in the first generation of resulting adults.

These two forms, even though they may be crossed successfully, should be regarded as distinct species based on adult characters and biological differences.

The successful cross-mating produced progeny in the first generation that emerged over the period of emergence of both parents—a few early when the pine form emerges, the remainder some two weeks later when the juniper form appears. In later years they emerge as the juniper form.

The juniper color pattern of the adults is dominant.

## HYLOTRUPES LIGNEUS, JUNIPER FORM. EXPERIMENT VIII

The adult forms generally included under *Hylotrupes ligneus* Fab. show a great variation of color patterns. Many of these varieties have been described as distinct species by Col. T. L. Casey. In the experiments conducted all color varieties, however, have been kept distinct only by the host in which they were found in nature and not by the color variations. The experiments were primarily conducted to test these variations in color patterns, but certain results bearing on the host-selection principle were obtained and are here described.

*Hylotrupes ligneus*, juniper form, has a wide selection of hosts. Specimens in the Forest Insect Collection of the Bureau of Entomology have been recorded from all genera of coniferous trees indigenous to North America. It uniformly prefers wood that has not seasoned a great deal.

Later winter or fall cuts in which the inner bark has remained sappy are most suitable. Species of wood which season more slowly, due to thick bark, must be cut earlier.

The time of first emergence varies greatly with locality, but the species is everywhere one of the first cerambycid beetles to fly in the spring. The larvæ in all cases feed immediately beneath the bark, only entering the sapwood in late summer to make a shallow pupal cell. Pupation and transformation to the adult usually take place in the fall.

The present experiment was started at Kanawha Station, W. Va. Dr. Hopkins felled a juniper (Juniperus) in October, 1914. This tree was infested the following spring and shipped to East Falls Church, Va. The colony has since been continued in juniper and one host strain has been produced in Douglas fir (Pseudotsuga). This particular color variety had never been recorded from Douglas fir.

PSEUDOTSUGA. EXPERIMENT VIII[1].—April 11, 1917, three females and two males were isolated on a piece of Douglas fir cut April 1. Eggs were laid and young larvæ entered the bark, but many died during the summer and only two constructed pupal cells. One adult was secured next spring. The fact that this wood was too green and that it seasons very slowly may have caused a higher mortality than would otherwise have occurred.

May 29, 1917, twelve larvæ and June 15, nine larvæ were transferred to this host, the wood then being better seasoned. March 1, 1918, five adults—four females and one male—were removed from pupal cells. The remainder of the larvæ had died. Two females and one male were used to continue the colony by caging on Douglas fir, cut in October, 1917. A good infestation was secured. A piece of juniper cut during January, 1918, had also been placed in this cage but was not infested. In January, 1919, four adults were removed from the logs—three males and one female. The remainder had all died and these were very weak and below normal size. One pair was recaged on juniper cut in January and Pseudotsuga cut in November. July 30, 1919, the sticks were examined, but no infestation was found in either wood.

### CONCLUSIONS

This juniper form of *Hylotrupes ligneus*, after feeding part of a year in a new host, showed a preference for the new host.

A high percentage of mortality occurred in producing the new host strain, which finally died out.

### HYLOTRUPES LIGNEUS, PSEUDOTSUGA FORM. EXPERIMENT XXXV

This form of *Hylotrupes ligneus* is much darker and more hairy than the preceding. It has been recorded only from Douglas fir. Its biology is essentially similar to that of the juniper form except that the adults emerge somewhat later. It is known from the Rocky Mountain region.

The colony was started from a small tree collected at Colorado Springs, olo., and shipped to Falls Church. This tree had been killed by Scolytus the fall of 1916 and infested by Hylotrupes in the spring of 1917.

In 1918 adults did not emerge until May and were caged on Douglas to continue the colony. They were recaged on Douglas fir in 1919, it all the larvæ died from a fungus attacking the bark.

### LOTRUPES LIGNEUS, PSEUDOTSUGA STRAIN VIII[1] AND PSEUDOTSUGA STRAIN XXXV

Two females from VIII[1] (the juniper form in Douglas fir) were held a cool cellar until adults of this XXXV variety emerged. May 29, 18, they were caged on Pseudotsuga with two males from the true ouglas fir form (XXXV). The two sexes absolutely avoided each other d were never observed to mate. The females died without laying gs. Many attempts were also made to mate the original juniper form th the Douglas fir form but without success.

### HYLOTRUPES LIGNEUS, SEQUOIA FORM. EXPERIMENT XL

The form of *Hylotrupes ligneus* occurring in sequoia is slightly larger it otherwise resembles that in juniper very closely, although the speciens reared in the experiments show a much greater variety of color ttern than do those from juniper.

April 2, 1918, a large series of these adults were removed from their ipal cells in *Sequoia sempervirens* (Lamb.) Endl. and isolated in small als by F. B. Herbert at Laurel, Calif. April 13, 1918, they were reived at Falls Church, Va.

JUNIPERUS. EXPERIMENT XL[1].—Three prominent color forms were ired and each was caged on a piece of juniper cut in January, 1918, ice no sequoia was on hand. They all oviposited, but about half of e larvæ died by July. The remainder made pupal cells and emerged. ie strain has since been continued in juniper.

PSEUDOTSUGA. EXPERIMENT XL[2].—April 20 one pair was caged on piece of Douglas fir cut in October, 1917. Eggs were laid and a better festation secured than with the juniper form (VIII). All larvæ died d no adults were secured in the spring of 1919. A fungus growth der the bark was responsible in a large measure.

### HYLOTRUPES LIGNEUS, JUNIPERUS STRAIN VIII × SEQUOIA STRAIN XL

April 13 several males from redwood (XL) were separately caged th females from juniper (VIII) held over in a cool cellar since they re isolated from the cells. One of these males mated with two females rst and third tried) immediately on being isolated with them. This ne male would not mate with the second female tried, nor would any iles of XL mate with females of VIII. Many juniper (VIII) males

were isolated with redwood (XL) females, but in no case did copulation take place.

The females of the juniper form mated with males of the sequoia form were caged on juniper cut in January, 1918, and good infestations were secured.

CONCLUSIONS ON THE ENTIRE HYLOTRUPES LIGNEUS GROUP

The experiments on the *Hylotrupes ligneus* group, as mentioned above were conducted primarily for the study of its color variation, and not a great deal of attention was devoted to the host-selection principle. The experiments cited show that among all the color varieties of this group there are probably two good species, the darker and more hairy Douglas fir form representing one species and all the other forms another. These two species absolutely refused to mate, but the varieties from sequoia and juniper were successfully crossed.

NEOCLYTUS CAPRAEA. EXPERIMENT VI

*Neoclytus capraea* is known to inhabit the eastern and central western United States, extending its range south and west into Arizona. It has been recorded from only two hosts, ash (Fraxinus) and white oak (*Quercus alba* of the Rocky Mountains). In the eastern United States it has never been found in oak. The condition of the wood necessary for oviposition by these beetles must be exactly right. It must have been freshly cut and the inner bark must be still moist and sappy. Should this inner bark be slightly dried the females will not oviposit on it unless forced to do so. Logs cut about two months before the flight period are preferred to older cuts or those cut during flight. Trees cut as early as November 15 are sometimes infested, but not commonly.

The adults fly very early in the spring in this locality (Falls Church, Va.), about the last week in March and the first two weeks of April. The larvæ feed chiefly in the wood proper. Mining beneath the bark for a short time, they then enter the sapwood and later the outer heartwood, extensively honeycombing it. Pupation and transformation to the adult take place in the early fall.

VI.—March 26, 1915, twelve adults were taken as they emerged from an ash log and were caged on freshly cut wood. A good infestation was secured, and the colony has since been continued in ash.

In the spring of 1919 no adults emerged. All the larvæ remained over as larvæ in their pupal cells until the fall of 1919, when they transformed to adults and emerged in 1920. No explanation for this can be offered unless the logs were too moist in the early part of the summer so that the larvæ did not develop properly. Excess humidity or excessive desiccation have both been found to produce retardation in development of larvæ in small isolated cages. This insect is one of the most regular of those reared, in the time of emergence and development of

the broods. Attempts have been made to start colonies in hickory and white oak.

HICORIA. EXPERIMENT VI[1].—March 31, 1917, seven adults (four females and three males) from ash were isolated on hickory cut February 1, 1917. No infestation occurred. May 31 of the same year fifteen larvæ, 2 to 4 mm. long, were transferred to hickory. Again on June 15, seven larvæ, 4 mm. long, were transferred to the same piece of wood. July 11 one larva was living and five more, over half grown, were transferred.

April 6, 1918, three adults (two males and one female) emerged from the hickory. They were caged on hickory and ash cut January, 1918. These adults were very weak and inactive, not at all characteristic of normal adults.

An examination in July showed neither wood to be infested.

July 24, 1918, twenty larvæ, one-half to three-fourths grown, were transferred from ash to hickory cut April 15, 1918.

April 11, 1919, one female emerged, one adult had died in its pupal cell, and the remainder of the larvæ had died before pupating. This female was mated with a male from ash and caged on hickory and ash of optimum cuts. An examination in July showed no infestation in the hickory, but the ash contained a few larvæ. These died later in the summer.

QUERCUS ALBA. EXPERIMENT VI[2].—April 1, 1917, four pairs of adults from ash were isolated on white oak cut in March, 1917. Eggs were laid on the wood, and the small larvæ bored through the bark, but all died before May 31. On this date fifteen larvæ, 2 to 4 mm. long, were transferred to white oak. July 11 one larva was living. September 17 all were dead.

In April, 1918, three pairs were caged on wood cut in January, 1918. July 18 many larvæ were still alive but under size. Several lived to pupate, but all died before the following spring.

SEASONING. EXPERIMENT VI.[a]—April 4, 1917, four pairs of adults were isolated on ash cut September 1, 1916, and white oak cut in March. The females laid eggs on the white oak, but the larvæ did not live. On May 31 neither wood contained larvæ.

## CONCLUSIONS

The foregoing experiments show that this species feeding in ash (Fraxinus) has become decidedly accustomed to that host. Several attempts, both by oviposition and larval transfers, to produce strains in *Quercus alba* Linn. and Hicoria have resulted in failure. In Hicoria the few adults secured were incapable of continuing the colony, and in both woods a high or total larval mortality occurred.

Even with this decided preference for a host, the adults laid eggs on a new host rather than on an unfavorable cut of the normal host.

MOLORCHUS BIMACULATUS. EXPERIMENTS IX, X, AND XXXVI

Two forms included under *Molorchus bimaculatus* Say have been caged in these experiments, a large form from hackberry (*Celtis occidentalis* Linn.), and a smaller form from dogwood (*Cornus florida* Linn.) and maple (Acer). They both are found throughout the eastern half of the United States. From the observations on the biology of these two forms they are regarded by the writer as distinct species. Both forms prefer early fall cuts of wood, but the Celtis form requires much drier seasoned material.

MOLORCHUS BIMACULATUS, CORNUS FORM. EXPERIMENT IX

The Cornus form feeds in a great variety of eastern hardwoods. It has been reared from Hicoria, Acer, Juglans, Quercus, Liriodendron, Cornus, Cercis, and Castanea. The larvæ feed beneath the bark, making a long, curved pupal cell in the wood. By September they have transformed to adults, which emerge early in May at Falls Church, Va. The flight is very regular, nearly all emerging at the same time. The adults are much smaller than those of the hackberry form.

In May, 1916, adults were reared from dogwood collected at Falls Church, Va. They were recaged on dogwood cut in April, but a poor infestation was secured from which only five adults emerged in 1917. These five adults were caged on September and November cuts of dogwood and redbud (*Cercis canadensis* Linn.). A good infestation occurred in the dogwood, but no larvæ were found in redbud.

May 1, 1917, five adults were isolated in a cage containing November cuts of dogwood and maple. The maple was not infested, but many larvæ were found in the dogwood.

In April, 1918, 1919, and 1920, the colony was continued only in dogwood. No selection tests were made.

MOLORCHUS BIMACULATUS, ACER FORM. EXPERIMENT XXXVI

This form in all respects is similar to the dogwood variety IX.

Infested limbs collected at Falls Church, Va., were caged in the summer of 1916.

May 1, 1917, five adults were caged on branches of maple and dogwood cut in September and November. The maple was infested but no larvæ entered the dogwood.

In 1918 many adults emerged from the maple and were recaged on October cuts of maple and dogwood. Eggs were laid on the maple, but the cage unfortunately was overlooked and became so dry that none of the eggs hatched.

MOLORCHUS BIMACULATUS, CELTIS FORM. EXPERIMENT X

The form in hackberry, in which the adults are much larger, has been reared only from this host. The larvæ feed as in the dogwood or maple

forms, but only about half the brood emerges at the end of the first year, the remainder going over in the larval stage to the following season. The adults emerge about a month earlier, April 1 to 10.

Infested hackberry branches from Hummelstown, Pa., were collected and sent to Falls Church, Va., in December, 1915, by J. N. Knull.

In April, 1916, 20 adults were caged on January cuts of hackberry, dogwood, and redbud, but only the hackberry was attacked.

In April, 1917, eight adults were isolated on sticks of redbud, dogwood, and maple, all cut in September and November. No eggs were laid in any of these woods. The form has since been continued in hackberry.

### CONCLUSIONS

A very decided predilection for the original host is exhibited by the host strains of this species. It is not surprising in the case of the hackberry form, as this is the only host from which it has been found. However, this form would not even lay eggs on any hosts other than the original. In the dogwood strain adults were not isolated on maple alone, nor were adults of the maple strain isolated on dogwood alone. If this had been done, it is very likely that infestations would have resulted.

### NEOCLYTUS ERYTHROCEPHALUS. EXPERIMENTS XI, XII, AND XIII

The adult and larva of *Neoclytus erythrocephalus* Fab. are quite different from those of *Neoclytus capraea*, but the range and habits are much the same. The species attacks wood in a greater variety of conditions, but the most favorable condition is an early spring cut. It has been collected in almost all eastern hardwoods.

The first flight occurs at Falls Church, Va., in late May or early June; consequently, that the wood may be sappy for infestation it must be cut during April. The species overwinters in the larva stage, pupation not taking place until early April. Farther south two or more generations occur each season.

Three host strains were collected in nature and experimented with.

#### NEOCLYTUS ERYTHROCEPHALUS, HICORIA FORM. EXPERIMENT XI

June 9, 1916, adults emerging from hickory at Falls Church, Va., were recaged on wood cut in late March. A good infestation was secured.

June 8, 1917, the colony was continued in April cuts of hickory. Two pairs were isolated in a cage containing hickory and redbud cut in April and dogwood and tulip (*Liriodendron tulipifera* Linn.) cut in May. In July an examination showed hickory to be the only wood infested.

May 23, 1918, two pairs were isolated on hickory, dogwood, and redbud cut on April 15. When examined on July 18 hickory was found to be lightly infested, dogwood heavily, and the redbud contained no larvæ.

May 24, 1918, six pairs of adults were caged on two pieces of hickory and one of dogwood, cut April 15, of the same size as those of the

experiment of May 23, 1918. When examined on July 18, both woods were heavily infested. Redbud was unintentionally omitted.

This experiment was repeated in 1919, two pieces of hickory, one of dogwood, and one of redbud being used. Two cages were prepared; in one, a single pair was isolated, the resulting infestation being, hickory heavily infested, dogwood and redbud uninfested; in the other cage three pairs were isolated, the resulting infestation being, hickory and dogwood both heavily infested, redbud uninfested.

NEOCLYTUS ERYTHROCEPHALUS, CORNUS FORM. EXPERIMENT XII

June 13 to 15, 1916, adults emerging from dogwood at Falls Church, Va., were recaged on this wood cut in April, 1916. A good infestation was secured. June, 1917, the colony was continued in dogwood and two pairs of adults were isolated in a cage containing dogwood and tulip cut May 30 and hickory and redbud cut April 18.

In July it was found that both redbud and dogwood contained few larvæ while hickory and tulip contained none.

For some unknown reason the larvæ continued in dogwood did not develop very well, and in 1918 only one female emerged. May 25, 1918, this female was mated with a male from hickory and isolated in a cage containing dogwood, hickory, and redbud cut April 15.

July 18, 1918, the dogwood was heavily infested, the redbud lightly, and the hickory contained one larva.

In June, 1919, one pair was caged on pieces of dogwood, redbud, and hickory. An examination in July showed dogwood to be very heavily infested, the redbud and hickory containing seven and six larvæ, respectively.

NEOCLYTUS ERYTHROCEPHALUS, CERCIS FORM. EXPERIMENT XIII

Redbud infested with this species was collected at Hummelstown, Pa., by J. N. Knull and sent to Falls Church, Va., in April, 1916. Adults emerged in June and the colony was continued in redbud. June, 1917, the colony was again continued in redbud, and two pairs of adults were isolated in redbud and hickory cut in April and tulip and dogwood cut in May.

An examination in July showed the redbud to be heavily infested; the dogwood and hickory contained several larvæ, and the tulip none.

In May, 1918, two pairs were again caged on redbud, dogwood, and hickory, all cut April 15. In July it was found that the redbud and the dogwood were heavily infested while the hickory contained but three larvæ.

The same experiment was repeated in 1919, and the results showed the redbud to contain eight larvæ, the dogwood five, and the hickory two.

The selection tests of 1917 were all carried out with the same quantity of wood; in each case the pieces were 1½ inches in diameter and 1 foot long. Each cage contained two pieces of the wood from which the

adults emerged and only one each of the others. This amount of the original host for two females was considered sufficient for oviposition without bringing in the quantity factor.

These adults are extremely active and run rapidly over logs when ovipositing in nature. They have very long hind legs. It was noticed that in the glass cylinder used for cages in 1918 these long legs were a disadvantage. The adults could not get a foothold on the glass and had difficulty in climbing up on the wood from the glass surface. They crawled awkwardly about and when coming in contact with any stick maneuvered until they managed to get on it. Such conditions may have influenced the wood selected, as the adults could only with difficulty go from one stick to another. In 1919 wire boxes were used, the wood lying flat on the bottom. In 1920 only ash and dogwood strains were continued.

CONCLUSIONS

These experiments up to 1919 did not seem to show results in any definite direction. Selections of the various host strains occasionally gave results in conformity with those generally obtained, while again just opposite results were recorded.

The experiments of 1919 showed results in closer conformity to those of other species. This may have been due to the different method of caging, which gave the adults more opportunity to move about and select the host.

LIOPUS ALPHA. EXPERIMENTS XXV AND XXX

Two color forms of *Liopus alpha* have been experimented with, a brown form from sumac (Rhus) and a gray form from hickory (Hicoria). These color forms are very distinct and easy to recognize as adults. They are not known from any other hosts. The sumac form has been collected throughout the eastern United States and as far west as the Rocky Mountains. The hickory form follows the range of the hickory trees.

The adults fly in late May and continue flying through June at Falls Church, Va. One year is required to complete the life cycle. The larvæ feed beneath the bark and pupate in the wood. They are found only in small branches.

LIOPUS ALPHA, RHUS FORM. EXPERIMENT XXV

The sumac form prefers branches cut in the early fall and dried standing in the air, although it will attack later cuts, provided they have dried considerably.

April 26, 1916, Mr. Champlain sent from Long Island, N. Y., a lot of infested sumac twigs which were caged at Falls Church, Va. In June the first adults emerged, and 20 were caged on sumac cut in November, 1915. Into the same cage were placed chestnut, hickory, and wild cherry twigs cut during the winter, but none of these latter woods were infested. Since then it has been continued in sumac.

CASTANEA. EXPERIMENT XXV[1].—September 20, 1916, fifteen larvæ, one-half to nearly full grown, were transferred from sumac to chestnut cut during March. July 10, 1917, one adult emerged, the only one from these transfers.

HICORIA. EXPERIMENT XXV[3].—July 25, 1916, eleven larvæ about half grown were transferred from sumac to hickory. August 9, eleven more were transferred. The larvæ seemed to do quite well and by winter many had made pupal cells.

During June, 1917, twelve adults emerged and were caged on pieces of hickory cut the preceding June, August, April, and February. No infestation occurred in any of the wood. The cage accidentally dried for a two-week period while the adults were ovipositing and this may account for the failure of infestation, as they require considerable moisture.

In June, 1917, adults from sumac were isolated in various cuts of hickory but no infestation occurred.

July 23, 1917, twenty-seven larvæ were transferred from sumac to hickory cut in September, 1916, and March, 1917. The larvæ did well and the following May and June 10 adults were reared and caged on hickory sticks cut in September, 1917. On several of these sticks bands of thin outer bark of sumac were tied.

The adults oviposited only on those sticks and at those places where the sumac bark was tied. July 30 they had not yet bored beneath the hickory bark proper, but by fall nearly all had entered the bark. Only one larva transformed to an adult in the summer of 1919. One adult emerged in 1920. Several larvæ did not transform but continued feeding beneath the bark during the summer of 1919.

LIOPUS ALPHA, HICORIA FORM. EXPERIMENT XXX

The Hicoria form was not successfully continued in confinement until the summer of 1917. It requires wood cut in August, dried in the air for a month or so, and then placed on damp earth over winter. In addition the adults must be well fed on fungus spores (*Endothea parasitica* was used) before they will oviposit.

It was again continued in hickory in 1918, 1919, and 1920. During June, 1919, many adults were caged on sumac branches and eggs were deposited. Three larvæ lived to construct mines under the bark, but these died before November.

## CONCLUSIONS

From the foregoing experiments and the fact that each of these two color forms has been taken only in the host given, it is evident that each has become restricted to that host and shows a strong predilection for it. Even after having fed for one year in a new host (Hicoria) adults developing from them showed a preference, in their oviposition, for that part of the hickory twig surrounded by Rhus bark. A fairly high mortality of larvæ occurred after transfer to the new host.

HYPERPLATYS MACULATUS. EXPERIMENTS XXVI, XXVIII, AND XXIX

*Hyperplatys maculatus* Hald. occurs throughout the eastern United States and west through the Rocky Mountain region. Two very similar species have been described, *H. maculatus* Hald. and *H. aspersus* Say, but the distinction is not drawn here, as each has many variations in color and maculation. It feeds on a great variety of hardwood deciduous trees. Probably any wood is attacked, provided it is in the proper condition for infestation. Smaller twigs and branches are usually preferred. Those that have died during the fall and lain on the ground so that a certain amount of fermentation has taken place in the bark give the optimum condition.

The larvæ feed entirely beneath or in the bark, only entering the sapwood to make a very shallow pupal cell. Adults fly in the early summer, late May, and early June. Two distinct variations occur in the length of the seasonal history. One form takes an entire year to complete the development, only one generation appearing each year. Another matures from one-half to three-fourths of the brood in August and September, the adults emerging and infesting new wood. This may be a basis on which to separate the two confused species. Four host strains have been experimented with, chestnut (*Castanea dentata*), gooseberry (Ribes), dogwood (*Cornus florida* Linn.), and yellow poplar (*Liriodendron tulipifera* Linn.).

HYPERPLATYS MACULATUS, LIRIODENDRON HOST STRAIN. EXPERIMENT XXVIII

The colony was started by collecting infested tulip branches in November, 1916, at Falls Church, Va. The following June adults emerged and were isolated in a cage containing yellow poplar, maple, dogwood, chestnut, and gooseberry cut in the fall of 1916. The original host, yellow poplar, was well infested, and a few larvæ were found in gooseberry, but no other woods were attacked. In 1918, 1919, and 1920 the colony was continued in yellow poplar; the selection was not repeated.

Only one generation of this form occurs each year.

CASTANEA. EXPERIMENT XXVIII[1].—June 4, 1917, ten adults from yellow poplar were caged on chestnut cut in November, 1916. A very good infestation was secured, forty-five adults emerging in 1918. Eight of these adults were isolated in a cage containing yellow poplar and chestnut cut in November, 1917.

Examination in August showed the yellow poplar to be heavily infested, while no larvæ were present in the chestnut.

June 1, 1919, two pairs from yellow poplar were isolated on chestnut, and in 1920 thirty-six adults emerged. Ten were caged on optimum cuts of chestnut, and the yellow poplar was heavily infested.

HYPERPLATYS MACULATUS, RIBES HOST STRAIN. EXPERIMENT XXVI

This colony was started in December, 1915, with infested gooseberry stems sent to Falls Church, Va., from Colorado Springs, Colo., by G.

Hofer. It has since been continued in gooseberry cut at Colorado Springs in the fall and shipped to Falls Church; in addition, several other host strains were produced. Only one generation of adults occurs each year.

May 20 to June 10, 1916, adults emerged and 47 were isolated in a cage containing gooseberry, chestnut, and wild cherry, all cut in the preceding fall. The gooseberry stems were heavily infested, a few larvæ were present in the wild cherry, but none were found in the chestnut. The colony has since been continued in gooseberry.

PRUNUS. EXPERIMENT XXVI[1].—The infested wild cherry twigs (described above) were caged separately, and in June, 1917, four adults emerged. These were caged again on a fall cut of wild cherry. The infestation was not very good, and only six adults were secured in 1918; these were recaged on the same wood, but no infestation occurred.

CASTANEA. EXPERIMENT XXVI[2].—As previously stated, the chestnut sticks were not infested in 1916 when caged with gooseberry. In June, 1917, nine adults were isolated on chestnut cut in November, 1916, and a good infestation was secured. June, 1918, nine adults emerged and were isolated in a cage containing chestnut and gooseberrry cut in November, 1917. Later examination showed only the gooseberry to be infested.

LIRIODENDRON. EXPERIMENT XXVI[3].—June 5, 1917, eight adults from gooseberry were isolated on tulip cut in November, 1916. Five adults emerged from these sticks in 1918 and were isolated in a cage containing tulip and gooseberry cut in November, 1917. Neither wood was infested.

HYPERPLATYS MACULATUS, CASTANEA HOST STRAIN. EXPERIMENT XXIX

In April, 1916, at Falls Church, Va., branches of chestnut (Castanea) containing larvæ in the pupal cells were collected and caged. Some of the adults emerging in June were isolated with chestnut cut in March, 1916, and the others isolated in a cage containing chestnut and dogwood (Cornus) branches cut in March, 1916. Those isolated on chestnut alone attacked this wood although it was a late cut. Those isolated on the two woods infested both, but the dogwood more heavily. Nothing more was done with the chestnut form. Many adults emerged that fall.

CORNUS. EXPERIMENT XXIX[1].—The dogwood sticks were then caged separately and adults secured in September, 1916, and more of them in June, 1917. Those emerging during the latter period were recaged on August and November cuts of dogwood, but no infestation occurred.

## CONCLUSIONS

In *Hyperplatys maculatus* host selection occurs to a certain degree; but this beetle behaves differently from most of the other species tested. Thus the tulip form (experiment XXVIII) in 1917 chiefly selected the same host, but it also oviposited on gooseberry. This gooseberry colony, however, was weak, and a high mortality in larvæ occurred.

Furthermore, although not selecting chestnut when the other host was present, they produced a good colony when isolated on it; but in 1918 these adults again selected tulip in preference to chestnut. The same was true with the original gooseberry form which was transferred to chestnut (experiment XXVI [2]), for in 1918 it returned to gooseberry in preference to chestnut.

SUMMARY OF RESULTS

(1) In practically all the species experimented with the adults show a marked predilection for the host in which they have fed as larvæ, provided they are not deterred by other factors, such as the unfavorable condition or the small quantity of the host.

(2) There is considerable variation in the degree of preference for the original host, as between different species. Thus—

(*a*) Certain species are capable of living in only one genus or species of plant, which consequently they select.

(*b*) Certain species, chiefly those living in nature in several hosts, can be forced to adopt a new host.

(*c*) Certain species, chiefly those feeding in nature in a great variety of plants, show little discrimination in the selection of hosts.

(*d*) Certain species feeding in nature in a great variety of hosts often show a preference for a few of these.

(3) In forced transference of individual adults of a species to a new host, a high mortality of the broods usually occurs, especially in the case of eggs laid by beetles emerging from the original host, in which case the mortality is often total. One-half to full-grown larvæ, however, usually can be successfully transferred to a new host and live and transform to adults.

(4) With some species that can be reared in a secondary (new) host, by the larvæ feeding one or part of one year, preference for that host is shown by the resulting adults.

(5) In general, the fewer the hosts in nature, the more marked the predilection for a particular host, and vice versa.

(6) Continued breeding in a given host intensifies the preference for that host.

(7) The condition of the host has a great influence on host selection, in that every species prefers an optimum condition of the host which it selects and will choose a new host in the optimum condition in preference to an old host in which the conditions are unfavorable.

(8) The quantity of wood at the disposal of the ovipositing adults may influence the insects in their choice between different kinds of host wood, in that, if there are many adults to a limited amount of the primary host, some species will select a secondary host if such is available. If this is done, however, the resulting brood is weakened.

It is altogether possible that these experiments may indicate the origin of certain closely related species or varieties of insects. For instance, a species restricted to a very few plants may accidentally be forced to

take a new host (as actually happened in the experiments with Cyllene in oak). A few individuals may survive and continue the strain so that it becomes, after a time, at least physiologically different and may also develop correlated differences of color or structure. It can hardly be said that such forms are much less distinct than in the case of the two species *Callidium antennatum* in pine and *C. janthinum* in juniper; for even though these have a slight color distinction and each is absolutely restricted to its own host, they interbreed. On the other hand, in the different forms of *Hylotrupes ligneus*, of which the eastern form in juniper is constant in marking, the western form in redwood is quite variable, as is also the Rocky Mountain form in Douglas fir. The juniper and redwood forms interbreed, but all attempts to mate either of these with the Douglas fir form have failed. All these forms can be furnished with substitute hosts, but in the experiments in which this has been done the original color pattern has resulted thus far.

The grape and hickory strains of *Cyllene pictus*, although showing no color differences, do not readily mate. Two species of Cyllene, *C. pictus* and *C. robiniae*, are separable only as adults, by a slight difference in the color pattern, yet in seasonal and biological habits they are strikingly different. It is conceivable that one of the two species originated through the adoption of a new plant and continuous breeding in that plant.

It may be asked, If one or two years' feeding in a new host results in individuals which prefer that host, thus giving rise at least to new physiological varieties, why does not this occur more frequently in nature? That it does occur must be granted, as we have species living in many host plants as well as those restricted to a species or genus, but that it is not of more common occurrence is believed to be due to the high mortality in first-stage larvæ in a new host rather than to absence of oviposition in the new host. Although the adults show a decided predilection for a favored host in ovipositing and even, in certain species, a preference for the plants in which the larvæ have fed for one or two generations, the instinct to oviposit seems to overbalance that of host selection, consequently new hosts are frequently selected—possibly more frequently in nature than is generally realized. As an example of this, take *Cyllene pictus* requiring hickory cut during the winter. This condition would be fully met in tops left during logging operations. When the timber cutting ceased, a concentration of adults would be left with none of the favored host plant available in the right condition. The grape, osage orange, and hackberry strains collected at Hummelstown, Pa., were in reality taken in a woods which had been logged for hickory and in which operations had ceased three years prior to the finding of these strains. At Falls Church, Va., in June, 1920, adults of *Neoclytus erythrocephalus* were observed ovipositing on pine logs. Much infested ash, from the previous year, was lying about from which they had emerged in great numbers.

# NOTES ON THE ORGANIC ACIDS OF PYRUS CORONARIA, RHUS GLABRA, AND ACER SACCHARUM

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During the study of other compounds found in the plants in question, we have incidentally isolated and identified the organic acids of the fruits of the wild American crab apple (*Pyrus coronaria* L.) and the smooth sumac (*Rhus glabra* L.). We have also made an examination of the product known as "maple sand" (found to be impure calcium malate) which is formed as a granular deposit in the pans during the process of boiling down sap of the sugar maple (*Acer saccharum* Marsh.) to make maple sirup. Every precise record of the distribution of plant products is distinctly worth while, and rather than hold our data on the acids of these three plants for incidental mention in papers dealing with other matters, we have thrown them together in the following notes.

## OCCURRENCE OF MALIC ACID IN PYRUS CORONARIA, AND ITS TRANSFORMATION INTO SUCCINIC ACID

As might have been predicted from the botanical relationship of *Pyrus coronaria* to the common apple, the very sour fruit of the American crab apple was found to contain malic acid. It was also found that in water extracts of this fruit, made without heat, there is a transformation of malic into succinic acid, apparently through the action of enzyms of the fruit itself. This discovery will be of no little interest if further investigations substantiate our belief that microorganisms were not concerned in the process.

Cold water extractions of crab apples collected near Ann Arbor, Mich., were made in the presence of both chloroform and toluol, with the expectation of obtaining solutions of the fruit acids free from pectin and other colloidal substances. The extractions were made in large stone jars, tightly packed with sliced fruits and filled to the top with water saturated with chloroform and toluol. At the bottom there was an excess of chloroform and at the top an excess of toluol. The solution quickly became intensely sour. The extraction was allowed to take place for several weeks, at the end of which time the apple tissues were as green and hard as when collected, showing no change whatever in appearance. The infusion was brown at the surface layer, but clear and of a pale straw color below. Only the non-oxidized lower part of the

solution was drawn off through a tubulature at the bottom of the jar. It was not until succinic acid instead of one of the usual fruit acids was isolated from this solution that suspicion arose as to the possibility of any fermentation, other than an autolytic one, having occurred, and it was then too late to examine the solutions for microorganisms. It can only be stated that there was no evidence that such were present. The high acidity would have prevented bacterial action, and the perfectly clear solution, saturated with chloroform and toluol, showed no evidence of the presence of yeasts.

The infusion was neutralized by stirring with calcium carbonate. During this process rapid darkening took place, with the formation, presumably, of the same brown oxidation product that forms when a cut surface of apple is exposed to the air. A dark precipitate separated, which was not a salt of one of the fruit acids. It was filtered off. From the filtrate it was possible to get relatively pure succinic acid by acidifying with hydrochloric acid and shaking with ether, but the more economical and easy procedure, by which a larger yield was obtained, is described below.

The neutralized filtrate was evaporated to a small volume and precipitated with several volumes of ethyl alcohol. An impure calcium salt separated as a sticky, molasses-like mass. It was dissolved in dilute hydrochloric acid, and the solution was concentrated until the succinic acid crystallized out. It was purified by crystallization from 10 per cent nitric acid, and finally by repeated recrystallization from water.

The pure acid thus obtained agreed in all properties with succinic acid. It melted at 184° to 185° C. (Rosenthaler (*13*)[1] gives 185° C.). The reaction mixture obtained by heating with concentrated sulphuric acid, when diluted, boiled, and neutralized with ammonia, gave a red solution with a strong green fluorescence, a characteristic reaction of succinic acid.

Combustions of the pure material dried at 110° C., resulted as follows:

(I) Weight of sample, 0.2378 gm.; $H_2O$, 0.1047 gm.; $CO_2$, 0.3556 gm.

(II) Weight of sample, 0.3063 gm.; $H_2O$, 0.1356 gm.; $CO_2$, 0.4530 gm.

Calculated for $C_4H_6O_4$; C, 40.66 per cent; H, 5.12 per cent.

Found: (I) C, 40.78 per cent; H, 4.94 per cent. (II) C, 40.33 per cent; H, 4.96 per cent.

Titration with sodium hydroxid (NaOH) gave the following results:

(I) 0.1776 gm. acid required 30.037 cc. *N/10* NaOH.

(II) 0.1453 gm. acid required 24.424 cc. *N/10* NaOH.

Calculated for $C_4H_6O_4$; replaceable H, 1.707 per cent.

Found: (I) 1.703 per cent; (II) 1.692 per cent.

A silver salt was prepared and analyzed, giving the following data:

(I) 0.4809 gm. salt gave 0.3119 gm. Ag.

(II) 0.4818 gm. salt gave 0.3126 gm. Ag.

Calculated for $C_4H_4O_4Ag_2$; Ag 65.02 per cent.

Found: (I) 64.86 per cent; (II) 64.88 per cent.

[1] Reference is made by number (italic) to "Literature cited," p. 228.

It will be observed that if malic acid had been present in the cold water extract, it would have been discarded with the mother liquor from the first crop of succinic acid crystals, since malic acid is not only very deliquescent but likewise difficult to crystallize from solutions containing sugars and other impurities. Some malic acid was doubtless lost at this point, but the large yield of succinic acid indicated that it was the chief acid derived from the fruits which had undergone autolysis.

It was of course a matter of interest to find out whether or not succinic acid was present also in the living fruit. A new supply of crab apples was therefore heated with water in an autoclave at 20 pounds pressure, and the juice, after filtration through cloth, was evaporated to a small volume and treated with several volumes of alcohol, to throw out pectin and other colloids insoluble in alcohol. The alcohol was distilled from the filtrate, which was concentrated, in vacuo, to a sirup. From this sirup it was impossible to obtain even a trace of succinic acid, by either of the methods which had been successfully used with the cold water extracts. It contained, on the other hand, a large quantity of malic acid, identified by the preparation and analysis of its silver salt. The concentrated sirup mentioned above was diluted with water, which brought about a separation of a small precipitate of red pigment, which was filtered off. When lead acetate was added to the filtrate, the acidity of the solution was so great that the first increment caused no precipitation of lead malate but did throw out a small amount of dark precipitate, which was of course removed. Further addition of lead acetate gave a voluminous precipitate of lead malate (A), which was filtered off and washed. A second yield of lead malate (B) was obtained from the solution by the addition of alcohol. The two precipitates were separately decomposed with hydrogen sulphid, neutralized with sodium hydroxid, and silver nitrate solution was cautiously added. The first few drops of the silver nitrate produced a dark precipitate which was removed by filtration. Further addition of silver nitrate caused white silver malate to separate. The precipitates were dried at 105 C. and analyzed as follows (two samples each from A and B):

(I) 0.5317 gm. salt from A gave 0.3315 gm. Ag.
(II) 0.7249 gm. salt from A gave 0.4518 gm. Ag.
(III) 0.3067 gm. salt from B gave 0.1882 gm. Ag.
(IV) 0.5374 gm. salt from B gave 0.3303 gm. Ag.
Found: (I) 62.34 per cent; (II) 62.32 per cent; (III) 61.36 per cent; (IV) 61.46 per cent.

Pure silver malate would have given 62.00 per cent silver. In view of the fact that the acid itself was not purified before the silver salt was formed, the analytical results are sufficiently close. Doubtless other acids than malic are present in very small quantity in the crab apple. The significant fact is that the fresh fruit contains malic acid as the predominant acid, and not enough succinic acid so that we were able to isolate it.

Although the old observations and experiments of Dessaignes (*7*) and of Liebig (*11*) showed that succinic acid was formed from malic acid when calcium malate was present in mixtures being fermented by yeast, nevertheless succinic acid is generally found in plants in such small quantities and always so intimately associated with asparagin that it is now customary to look upon it as a degradation product of protein rather than as directly related in metabolism to the other plant acids. The possibility of amino compounds giving rise by enzym action in the plant to succinic acid is sufficiently indicated by such discoveries as that of Ehrlich (*8*), who has traced the production of succinic acid by yeast to the fermentation of glutamic acid, and of Harden (*10*), who has shown that putrefactive bacteria (*Bacillus coli communis*), in the presence of glucose, will transform aspartic acid almost quantitatively into succinic acid. However, it must be stated that the whole subject of the place of succinic acid in metabolism is much in need of investigation. It would be a decided step forward to show that it is possible for malic acid to be transformed directly into succinic acid by enzym action, as appears to have taken place by autolysis in the crab apple.

We wish to indicate the possibility that such a transformation takes place and to point out that green fruits containing malic acid afford ideal material for a study of the problem. We do not wish, however, to give the impression that the possibility of fermentation by microorganisms was absolutely excluded in our work.

To anyone who may be inclined to take up the problem of acid transformations in green fruits, a word of caution may not be amiss with regard to the statements that have crept into general reference books such as those of Czapek (*6, p. 434*) and Wehmer (*14*) with regard to the distribution of succinic acid in plants. It is recorded from a number of unripe fruits but has actually been isolated or satisfactorily identified in very few cases. In 1876 Brunner and Brandenburg (*2*) isolated it from the juice of unripe grapes (*Vitis vinifera* L.). The source of most subsequent reports is a paper published in 1886 by Brunner and Chuard (*3*). These authors called attention to the earlier observation of Buignet (*4*) that the juice of green fruits is capable of absorbing a large amount of iodin, which enters into chemical combination with some constituent of the juice. At the same time, a precipitate is formed, which Buignet erroneously supposed to be the iodin compound. Brunner and Chuard, taking up the problem at this point, showed that the iodin compound remained in solution; and they obtained evidence which satisfied them that it was a glucosid of monoiodosuccinic acid, derived from a naturally occurring glucosid of succinic acid. Their investigation covered a considerable number of green fruits and plant juices; and they actually isolated succinic acid, as such, from unripe gooseberries and from the petioles of rhubarb. In the other instances it was merely inferred from analogy that the supposed succinic acid glucosid was present. The procedure

was to treat the juice with lead acetate, which supposedly threw out all iodin-absorbing compounds except the succinic acid glucosid. Then the presence of the latter, which was never isolated at all, was inferred from two circumstances: (*1*) that the purified juice absorbed iodin, and (*2*) that, after the absorption of iodin, a precipitate could be obtained with basic lead acetate, supposed to be lead monoiodosuccinate, which when treated with a mineral acid to liberate the free monoiodosuccinic acid, and then with finely divided metallic silver, gave malic acid. The production, under these circumstances, of malic instead of tartaric acid was thought to indicate that iodosuccinic acid had been present rather than an iodin derivative of the widely distributed malic acid.

The weakness of the whole argument is sufficiently obvious without going into detail, since neither the putative glucosid of succinic acid nor the iodosuccinic acid was isolated; and it was not shown that the basic lead acetate precipitate was free from lead malate, which one would naturally expect to be found there. To the physiologist who is interested in the ripening of fruits it will be clear that the whole problem of the distribution and significance in metabolism of succinic acid is much in need of more study. Especially, there can be no doubt that Buignet's iodin-absorbing compound (*4*), whatever it may be, should be taken account of in studies of fruit ripening. It exists in large amount in the unripe fruit and disappears as ripening proceeds. As far as we are aware, it is not even referred to in the recent literature of the subject.

## ACIDS OF RHUS GLABRA

The acid of the sour, red pericarp of the sumacs (several species related to *Rhus glabra*) has been variously reported by different investigators as citric, malic, and tartaric. Gallic acid has likewise been reported. The closely related species of true sumacs are doubtless alike as to acid content. Our work, confined to *R. glabra*, has verified the findings of Rogers (*12*) nearly a century ago, and Frankforter and Martin (*9*) that the fruit acid is malic, nearly all in the form of the acid calcium salt. We were also able to isolate free gallic acid, which seems not to have been reported from this particular species. There are statements in the older literature that free gallic acid occurs in the leaves of the European sumac, *R. coriaria* L.

The berries of *Rhus glabra* were boiled with successive quantities of distilled water. The water solutions were clarified and largely freed from tannin by boiling with hide powder and egg albumen, and were then shaken with ether. The combined ether extracts were evaporated to a sirupy consistence and deposited gallic acid as a yellow powder. The latter was filtered off on a Buchner funnel and crystallized repeatedly from water. It was obtained in pure and almost colorless condition by precipitation from solution in absolute alcohol by chloroform, or by recrystallization from glacial acetic acid. As obtained by crystallization

from water it formed brown aggregates of large crystals containing one molecule of water. (Calculated for $C_7H_6O_5.H_2O$, $H_2O$, 9.57 per cent; found, 9.39 per cent.) It was identified by the usual tests. Mr. N. A. Lange made combustions of some of the purified acid and of its triacetyl derivative, the results of which he kindly permits us to publish as follows:

I. The acid gave C, 50.19 per cent; H, 3.92 per cent. Calculated for gallic acid: C, 49.40 per cent, H, 3.56 per cent.

II. The acetyl derivative gave C, 53.91 per cent; H, 4.13 per cent. Calculated for triacetyl gallic acid, C, 54.39 per cent; H, 4.06 per cent.

The melting point of the triacetyl gallic acid, stated variously in the literature from 151° to 165° and 166° C., was 162° to 163° C.

After the removal of tannin and gallic acid the aqueous extract from the berries was largely neutralized with calcium carbonate and filtered hot, after considerable concentration. Alcohol threw out a voluminous precipitate, the first fractions taffy-like, later ones solid. These fractions were treated with enough hydrochloric acid to form the acid calcium salt, and were repeatedly treated with animal charcoal and recrystallized from hot water.

The pure crystals were dissolved in water, exactly neutralized with standard alkali; and normal silver malate was precipitated by the addition of silver nitrate. The four successive fractions of the crude calcium salt were designated A, B, C, and D, and each was purified and converted into the silver salt. In addition, a portion of fraction A was purified by further recrystallization and was obtained in two portions called Aa and Ab, from which silver salts were also prepared. The duplicate analytical figures for all of the silver precipitates are given in Table I.

TABLE I.—*Duplicate analyses of silver salts prepared from a series of precipitates obtained by fractional separation with alcohol from an aqueous solution of calcium salts of the organic acid of the sumac fruit*

| Fraction. | Weight of silver salt. | Weight of silver. | Percentage of silver. |
|---|---|---|---|
| A-I | 0.2895 | 0.1794 | 61.96 |
| A-II | .2133 | .1318 | 61.79 |
| Aa-I | .4385 | .2721 | 62.05 |
| Aa-II | 1.1358 | .7048 | 62.05 |
| Ab-I | .6137 | .3794 | 61.82 |
| Ab-II | .9674 | .5962 | 61.63 |
| B-I | .4541 | .2806 | 61.79 |
| B-II | .6359 | .3932 | 61.83 |
| C-I | .4242 | .2624 | 61.85 |
| C-II | .5015 | .3099 | 61.79 |
| C-III | .3921 | .2420 | 61.71 |
| D-I | .4195 | .2596 | 61.88 |
| D-II | .5263 | .3259 | 61.92 |

The figures from all the fractions are in excellent agreement with each other and agree fairly well with malic acid. The results prove beyond much doubt that only one acid is present in any quantity. Rogers (*12*), the first to show the presence of calcium malate in berries of *Rhus glabra*, did not attempt to prove that malic acid was the only one present, and subsequent work was less careful than his. Although convinced by the identity of the silver salts that nothing but malic acid was present in our material, we felt that the determinations should be closer to the calculated value. The average of 13 determinations makes the percentage of silver in the silver salt 61.85, whereas the theoretical value is 62.00 for pure malic acid. We, therefore, prepared silver malate, using a Kahlbaum preparation of the acid, and made four silver determinations in the same manner in which our other determinations were made. The four determinations gave us 61.94 per cent, 61.92 per cent, 61.81 per cent, and 61.91 per cent, averaging 61.89 per cent silver in pure silver malate by our method of preparation and analysis. There can, therefore, remain no doubt that the acid of sumac berries is all malic.

## MALIC ACID IN SUGAR-MAPLE SAP

It is no new observation that malic acid is present in the sap of the sugar maple. Cowles (*5*), for example, has published methods for the estimation of malic acid in maple products. Although it might have been anticipated that the granular precipitate known as "maple sand" which is deposited in the pans during the concentration of the sap would prove to be calcium malate, no one, as far as we know, has previously reported an analysis. Bloor (*1*) used "sugar sand" as a source of acid in his work on the transformation of malic acid into sugar by the tissue of the maple[1] but gave no data to bear out the natural and perhaps quite justifiable inference that the acid was actually malic. Our sample was kindly obtained for us from Ohio, by Dr. Clinton A. Ludwig, now of Clemson College, S. C. It was only necessary to add to the "maple sand" sufficient hydrochloric acid to transform the crude calcium malate into the acid calcium salt. The latter was obtained pure by repeated boiling with animal charcoal and recrystallization from hot water. It was neutralized with alkali, and silver nitrate was added to precipitate the insoluble silver malate. Three separate analyses for silver gave the following results:

(I) 0.4129 gm. silver salt gave 0.2560 gm. Ag.
(II) 0.1922 gm. silver salt gave 0.1190 gm. Ag.
(III) 0.2892 gm. silver salt gave 0.1796 gm. Ag.
Calculated for $C_4H_4O_5Ag_2$; Ag, 62.00 per cent.
*Found: (I) 62.00 per cent; (II) 61.91 per cent; (III) 62.10 per cent.*

[1] It may be noted that Bloor used tissues of "Acer saccarinum" for his work. Since he gives no authority for the name, one is left in doubt as to whether he means the silver maple (*A. saccharinum* L.) or the sugar maple (*A. saccharum* Marsh.; *A. saccharinum* Wang., not L.).

SUMMARY

(1) The acid of the sour fruit of the wild American crab apple, *Pyrus coronaria*, is malic acid. When the fruit undergoes autolysis under anaerobic conditions, in the presence of chloroform and toluol, this acid appears to be transformed largely into succinic acid. Further experiments, however, will have to be made in order to repeat the observations and to determine the exact process involved.

(2) The acid of the outer part of the red fruit of the smooth sumac, *Rhus glabra*, is malic acid, occurring in the form of the acid calcium salt. With it is associated a considerable quantity of free gallic acid.

(3) Malic acid is present in the form of calcium salts (both acid and normal) in maple sap. The product known as "maple sand" obtained from the evaporating pans is crude calcium malate.

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# FERTILITY IN SHROPSHIRE SHEEP[1]

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Heape (*4*)[2] mentions that in some breeds young ewes bear fewer twins than older ewes. Carlyle and McConnell (*2*) reported some observations which they had made on the effect of age on fertility in sheep from which they concluded that ewes from 3 to 6 years old averaged a larger percentage of lambs than younger or older ewes, and also that 1-year-old rams were not so prolific as those 2 or 3 years old. The same conclusions were reached by Humphrey and Kleinheinz (*6*) from a study of later records of the Wisconsin flock. Recently Jones and Rouse (*7*) showed that in sheep the percentage of twins increased with age until 5 years, when there was a decided drop.

The present paper gives the results of a study of the influence of age and season upon fertility in American Shropshire sheep.

The source of data is the American Shropshire Sheep Record (*1*). Individuals with registry numbers between 325502 and 344869 have been used, date of birth noted, whether born as single, twin, or triplet, and age of dams and sires looked up.

## AGE OF EWE AND FERTILITY

Table I shows the percentage of lambs born as singles, twins, and triplets from dams of various ages. Ewes under 1 year and 6 months are grouped in the 1-year class, those 1 year and 7 months to 2 years and 6 months in the 2-year class, and so on. The percentage in multiple births increases to 4 years and remains fairly constant through 8 years. For the older groups the numbers are too small to draw conclusions.

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[1] Paper No. 16 from the Laboratory of Genetics, Agricultural Experiment Station, Urbana, Ill.

[2] Reference is made by number (italic) to "Literature cited," p. 234.

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TABLE I.—*Age of ewe and fertility*

| Age of dam in years. | Total number of offspring. | Percentage of singles. | Percentage of twins. | Percentage of triplets. | Percentage in multiple births. |
|---|---|---|---|---|---|
| 1 | 370 | 77.0 | 23.0 | ........ | 23.0 |
| 2 | 2,299 | 66.4 | 33.2 | 0.4 | 33.6 |
| 3 | 2,025 | 63.6 | 36.1 | .3 | 36.4 |
| 4 | 1,762 | 57.6 | 41.4 | 1.0 | 42.4 |
| 5 | 1,256 | 58.0 | 43.0 | 1.0 | 44.0 |
| 6 | 942 | 53.7 | 46.0 | .3 | 46.3 |
| 7 | 506 | 56.3 | 43.1 | .6 | 43.7 |
| 8 | 405 | 54.8 | 44.5 | .7 | 45.2 |
| 9 | 157 | 62.4 | 37.0 | .6 | 37.6 |
| 10 | 96 | 38.5 | 61.5 | ........ | 61.5 |
| 11 | 23 | 60.9 | 39.1 | ........ | 39.1 |
| 12 | 3 | 100.0 | ........ | ........ | ........ |
| 13 | 4 | 50.0 | 50.0 | ........ | 50.0 |
| 14 | 1 | 100.0 | ........ | ........ | ........ |
| 15 | 4 | 75.0 | 25.0 | ........ | 25.0 |
| 16 | 5 | 20.0 | 80.0 | ........ | 80.0 |
| 20 [a] | 1 | 100.0 | ........ | ........ | ........ |
| | 9,868 | 60.8 | 38.7 | .6 | 39.2 |

[a] This may be a mistake in the record.

## AGE OF RAM AND FERTILITY

Table II gives the percentages of lambs born as singles, twins, and triplets born from sires of various ages. From these percentages one can not ascribe to the ram any influence on fertility. Carlyle and McConnell (*2*) thought that 1-year-old rams were not so prolific as older rams, but this is not borne out by the figures in Table II.

TABLE II.—*Age of ram and fertility*

| Age of ram in years. | Total number of offspring. | Percentage of singles. | Percentage of twins. | Percentage of triplets. | Percentage in multiple births. |
|---|---|---|---|---|---|
| 1 | 1,101 | 58.7 | 40.4 | 0.9 | 41.3 |
| 2 | 3,265 | 60.6 | 39.0 | .5 | 39.4 |
| 3 | 2,552 | 59.1 | 39.9 | 1.0 | 40.9 |
| 4 | 1,460 | 65.8 | 33.8 | .2 | 34.0 |
| 5 | 650 | 55.5 | 43.8 | .6 | 44.5 |
| 6 | 434 | 66.1 | 33.6 | .2 | 33.9 |
| 7 | 244 | 70.5 | 29.5 | ........ | 29.5 |
| 8 | 118 | 74.6 | 25.4 | ........ | 25.4 |
| 9 | 71 | 63.4 | 36.6 | ........ | 36.6 |
| 10 | 47 | 68.1 | 25.5 | 6.4 | 31.9 |
| 11 | 2 | 100.0 | ........ | ........ | ........ |
| 12 | 3 | 100.0 | ........ | ........ | ........ |
| | 9,947 | 61.2 | 38.2 | .6 | 38.8 |

## TIME OF BIRTH AND TWINNING

Heape (*5*), who gathered information from flock masters, states that 55 per cent of them reported that twins were usually born early in the

lambing season. To test this point Table III was made, showing the month of birth and the percentages of singles, twins, and triplets. It is readily seen that a larger percentage of twins is born early in the season than is born later. Of the 3,790 lambs born in January, February, and March 42.3 per cent are twins, while of the 4,617 born in April, May, and June only 36.1 per cent are twins. If the triplets are added in with the twins the percentages are 43.1 in multiple births for January, February, and March, and 36.7 for April, May, and June. As Heape (5) points out, this may be due to the ewes with the most vigorous and active generative systems coming into heat earlier in the season. This may be also affected by the fact that early in the breeding season more green feed is available, a factor influencing the number of twins produced.

TABLE III.—*Months of birth (Shropshires)*

| Month. | Total number. | Percentage of singles. | Percentage of twins. | Percentage of triplets. |
|---|---|---|---|---|
| January | 33 | 75.8 | 24.2 | ........ |
| February | 471 | 56.7 | 43.1 | 0.2 |
| March | 3,286 | 56.7 | 42.4 | .9 |
| April | 3,615 | 62.4 | 37.0 | .6 |
| May | 966 | 66.3 | 32.9 | .8 |
| June | 36 | 75.0 | 25.0 | ........ |
| August | 2 | 100.0 | ........ | ........ |
| September | 1 | 100.0 | ........ | ........ |
| December | 7 | 100.0 | ........ | ........ |

In the hope that additional information might be obtained, a study was made of the Dorset breed (3), which produces a large number of young in the fall. Table IV gives the month of lambing and the percentages of singles, twins, triplets, quadruplets, and of all multiple births.

TABLE IV.—*Months of birth (Dorsets)*

| Month of birth. | Total number. | Percentage of singles. | Percentage of twins. | Percentage of triplets. | Percentage of quadruplets. | Percentage in multiple births. |
|---|---|---|---|---|---|---|
| January | 1,818 | 61.5 | 37.1 | 1.3 | 0.1 | 38.5 |
| February | 2,386 | 54.3 | 41.9 | 3.8 | ........ | 45.7 |
| March | 3,919 | 52.7 | 43.9 | 3.2 | .2 | 47.3 |
| April | 2,366 | 51.7 | 45.4 | 2.6 | .3 | 48.3 |
| May | 857 | 54.8 | 43.1 | 2.1 | ........ | 45.2 |
| June | 296 | 59.8 | 38.5 | 1.7 | ........ | 40.2 |
| July | 90 | 65.6 | 27.8 | 6.6 | ........ | 34.4 |
| August | 102 | 68.6 | 30.4 | 1.0 | ........ | 31.4 |
| September | 925 | 73.7 | 25.3 | .9 | .1 | 26.3 |
| October | 1,546 | 66.2 | 32.9 | .9 | ........ | 33.8 |
| November | 1,088 | 67.7 | 30.5 | 1.8 | ........ | 32.3 |
| December | 1,418 | 61.0 | 36.8 | 2.2 | ........ | 39.0 |
| | 16,634 | 57.8 | 39.7 | 2.4 | .1 | 42.2 |

From Table IV it can be clearly seen that the percentage of multiple births is greater in the spring. If the births occurring from February to June, inclusive, are combined it is found that 48.2 per cent are in multiple births, while for the other months the percentage is 34.9. In Shropshires a larger percentage of twins or multiple births occurs in January, February, and March than later. This condition does not seem to hold for the Dorsets. Therefore, this condition in the Shropshires is not likely due to more green feed early in the mating season. The causes of these significant differences in multiple births at different seasons among sheep are yet to be discovered.

## SUMMARY

(1) Multiple births increase with age up to 4 years. From this point they remain fairly constant until 8 years. Beyond this age the numbers are too small to draw conclusions.

(2) The age of the ram has no influence on the percentage of multiple births.

(3) Among Shropshire sheep more multiple births occur early in the lambing season than later.

(4) Among Dorsets more multiple births occur in spring than in fall.

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# RELATION OF SOIL TEMPERATURE AND OTHER FACTORS TO ONION SMUT INFECTION

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## OCCURRENCE OF ONION SMUT IN RELATION TO CLIMATE AND CULTURAL PRACTICES

The onion smut fungus, *Urocystis cepulae* Frost, was first reported by Ware (*11*)[1] in the Connecticut River Valley in 1869. At this early date it was causing some injury to the onion crop, and in 1888 it was reported by Thaxter (*10*) to be of much importance in the old onion soils of southern New England. During the years which have since elapsed it has successively appeared and become an economic factor in nearly all the more westerly regions of intensive onion culture of the northern States, from New York to Oregon. It is possible that this fairly rapid distribution of the parasite has been occasioned to some extent by smut spores carried with the seed, as already noted by Chapman (*2*) and Munn (*7, p. 412*). It has, however, more probably been brought about by the increasingly widespread distribution of onion sets. Many of these sets are grown in the northern States on smut-infested soils, and since they are shipped in quantity to all parts of this country, and even exported, their rôle in the wide dissemination of smut spores is obvious.

Chance introduction of the smut fungus in this way in the northern commercial onion-growing sections seems almost certain to lead to its permanent establishment. This evidently results from the fact that the common intensive practice of continuous cropping with onions for an indefinite term of years tends, when once the inoculum is introduced in the soil, to favor its increase and wider distribution season by season until it becomes a factor limiting further success with this crop. While this holds true for the northern States, it does not seem to be so in the southern sections. This is the more noteworthy since northern sets grown on smutty soil are each year shipped into the southern onion districts for propagation. This regional limitation of the smut fungus was impressed upon one of the authors (Walker) in connection with a

[1] Reference is made by number (italic) to "Literature cited," p. 261.

survey which he made some two years ago of the chief onion-growing centers of the entire country, as a representative of the Office of Cotton, Truck, and Forage Crop Disease Investigations of the United States Department of Agriculture. In connection with this, he personally inspected the leading onion-growing sections of Texas and Louisiana and conferred with the plant pathologists of these two States, Drs. C. W. Edgerton and J. J. Taubenhaus. No evidence of the disease was found, and it had not been reported to the Experiment Station of either State.[1]

In comparing the distribution and occurrence of onion smut in different sections of the country, it is necessary to keep in mind that two distinct types of onion culture are practiced in the United States. The first is followed in practically all of the northern sections, the second is the rule in the southern commercial growing regions, and in one or more sections in the Pacific coast States. In the first, or northern, type the seed is sown directly in the field as early in the spring as the soil can be properly prepared—that is, in March, April, or May, according to local climatic conditions. The bulk of the crop is harvested in these northern districts in August and September. The Globe varieties predominate, including the red, yellow, and some white. In the second, or southern, type of culture the seed is sown in special beds in late summer. The seedlings are then transplanted to the main field during the early winter months and the crop is harvested during the period from April to July. Here the Bermuda, Italian, and Spanish varieties predominate. The survey previously referred to brought out the fact that onion smut has become established in essentially all of the older onion-growing sections, which practice the first type—with spring sowing of seed—while smut is either entirely unknown or of no economic importance in those localities where the seed is sown in summer followed by transplantation. Wherein lies the explanation? As already suggested, it can not be due to the matter of chance introduction of the organism. This is certainly being distributed frequently and widely throughout the South. It would seem rather to be associated with some of the factors incident to the southern type of culture. The conspicuously different factors as outlined above are three: (1) The varieties used, (2) transplantation vs. direct seeding, (3) climatic differences associated with season of culture.

Greenhouse experiments, in which we have tested the different types, have shown that the Bermuda and Spanish varieties which are used in the South are as susceptible to smut infection as are the Globe varieties of the northern sections. Hence varietal resistance does not furnish the explanation. Turning to cultural methods, we find that in the South the seed beds in which the onions are grown preliminary to transplantation are usually of considerable size and are located as a rule in a portion of

[1] The authors are indebted to Doctors Edgerton and Taubenhaus for continued cooperation in the search for the smut in their respective States. They each reported again early in the current year that not a single specimen had as yet been found.

the main field. Therefore, if the organism were present and environing factors were favorable, it does not seem probable that this method of culture would completely inhibit the disease. Indeed, judging from our experience with cabbage transplantation in relation to clubroot and other soil- or seed-borne diseases, this method, instead of reducing the trouble, is likely to serve as a ready means of distributing the parasites with diseased seedlings from localized centers to wider areas. We are thus forced to turn for explanation of the absence of smut in the South to the third suggestion, that relating to climatic differences, bearing in mind the respective cultural seasons. The most evident environmental differences associated with the two types of culture relate to soil temperature and moisture during the time of seed germination and early seedling development, which constitute the smut infection period. In the northern type, the spring-sown seed develops in a soil which is comparatively cool and which has in general a relatively high and constant surface moisture content. In the southern type, the summer-sown seed must germinate and pass the early developmental stages in a soil of relatively high temperature and subject to superficial desiccation. Our problem has, therefore, necessitated an attempt to analyze and evaluate the possible factors associated with variations in soil moisture and soil temperature during the seedling stage.

## INFECTION PERIOD

It has been of obvious importance in this study to know quite definitely the period in the development of the host at which infection actually occurs. Thaxter (*10*) gave critical attention to the time and manner of infection, concluding that the fungus always invaded the young seedlings below the surface of the soil and that, by subsequent growth of the host, the infected cells were commonly carried above the ground before visible signs of the disease appeared. He also noted that onion sets and onion bulbs replanted for seed growing were not attacked and suggested that the seedling was probably subject to attack in only the early stages of its development. Sturgis (*9*) later found that seedlings half as thick as a lead pencil and about 5 inches high, transplanted into smutty soil did not contract the disease. Sirrine and Stewart (*8*), in an experiment started at Jamaica, N. Y., on May 2, sowed eight rows of onion seed, each 10 feet in length, in soil free from smut. Alternate rows were left as controls. Soil from a smut-infected field was introduced in three ways: (1) in the furrow with the seed in two rows; (2) on the surface of the soil after the furrow was closed in one row; and (3) in a fourth row, on the surface of the soil 11 days after planting, this being shortly after the seedlings appeared above ground. The control rows remained healthy. Heavy infection occurred with the first treatment, slight infection with the second, no infection with the third. It seems possible

that in the third treatment, where the inoculum was merely placed on the surface of the soil when the seedlings were well started, the method failed to insure a sufficiently intimate and immediate contact of germinating spores with embryonic tissue to justify definite conclusions. Reviewing the evidence as a whole, however, it is obvious that the smut fungus is capable of invading the onion seedling for only a short period after seed germination.

In order to define more exactly the limits of this period of smut infection, we carried through a series of greenhouse trials. In the first of these 17 pots of sterilized greenhouse soil were planted with Red Globe onion seed which had been treated with 1 to 25 formaldehyde solution for 15 minutes. At two-day intervals beginning the eleventh day after planting, two pots were inoculated by mixing smut-infested soil in the upper layers of the pot, so that the inoculum was brought into close contact with the embryonic region of the cotyledon. At the time of inoculation, all retarded seedlings were removed, so that only plants of uniform height were considered in each case. All plants were pulled and examined for signs of the disease three to four weeks after inoculation. The results of this experiment, given in Table I, show that, under greenhouse conditions at least, infection may occur until the cotyledon is about 2½ inches above ground, or for a period of two weeks or more after sowing. Thus, the infection period appears to be slightly longer than that reported by Sirrine and Stewart (*8*).

TABLE I.—*Relation of the stage of development of the onion seedling to infection by Urocystis cepulae*

| Soil treatment. | Pot No. | Length of period between sowing and inoculation. | Height of cotyledons above ground at inoculation. | Number of plants. | Percentage infected. |
|---|---|---|---|---|---|
| | | *Days.* | *Inches.* | | |
| Inoculated | 1 | 11 | 1–1½ | 28 | 89 |
| | 2 | 11 | 1–1½ | 18 | 67 |
| | 3 | 13 | 1½–2 | 24 | 21 |
| | 4 | 13 | 1½–2 | 19 | 26 |
| | 5 | 15 | 1½–2 | 17 | 53 |
| | 6 | 15 | 1½–2 | 16 | 75 |
| | 7 | 17 | 2 –2½ | 19 | 16 |
| | 8 | 17 | 2 –2½ | 20 | 25 |
| | 9 | 19 | 2½–3 | 11 | 00 |
| | 10 | 19 | 2½–3 | 11 | 00 |
| Uninoculated | 11 | | | 14 | 00 |
| | 12 | | | 37 | 00 |
| | 13 | | | 22 | 00 |
| | 14 | | | 21 | 00 |
| | 15 | | | 18 | 00 |
| | 16 | | | 25 | 00 |
| | 17 | | | 45 | 00 |

The foregoing experiment was repeated in a somewhat cooler house, in which the temperature remained close to 15° C. most of the time, rising to about 20° during the middle of the day. Under these conditions, the maximum length of the cotyledon was about 2¼ inches. The data from this experiment are reported in Table II. The plants became immune at approximately the same time as noted in the first experiment—between the nineteenth and twenty-fourth days after sowing, when the cotyledon had about attained its full growth and as the first leaf was emerging. It will be recalled that the basal portion of the cotyledon, as with each of the later leaves, forms a collar or sheath inclosing the lower parts of the younger leaves. The question arose as to whether or not immunity to smut infection is directly associated with maturity of the tissues. If so, it would seem that the explanation of this later escape of the onion plant from infection lies in the fact that the maturing basal sheath forms a thin but normally complete barrier of resistant tissue between the potentially infective soil and the deeper-lying embryonic tissues of the younger developing leaves. The removal of this mechanical barrier might, therefore, permit of infection at a later stage. In order to determine whether this is the case, the following experiments were undertaken. After the thirty-first day, when the onion seedlings had passed the so-called susceptible period, the cotyledons were carefully removed from the plants in one pot, and infected soil was placed around the base of the exposed first leaf. Sixty per cent of the plants thus treated became infected as shown in Table II, pot 9. This proves that the first leaves are susceptible even after the cotyledon becomes immune. On the fortieth day, a 1-inch layer of infested soil was placed on top of pots 10 and 11, so as to surround the first leaves in proximity to the axils. Pot 10 was left at the same temperature (15° to 20°) for 24 days and pot 11 was removed to a temperature of 25° to 28° for the same period. About 5 per cent of the plants in pot 10 showed infection of the first leaf as compared with 28 per cent in pot 11. The reason for the increased percentage of infection at the higher temperature has not been satisfactorily explained. It may simply have been consequent upon the stimulated growth of the onion foliage. However this may be, it is evident that the basal portion of the first leaf remains susceptible to infection for a short time, at least, after it emerges from the cotyledon.

From a summary of these results it appears that our own experimental data regarding the duration of the period of infection agree in the main with those of previous investigators. The conclusion seems justified that disease resistance is correlated with tissue maturity, and that the onion cotyledon becomes immune to smut infection at about the stage when growth ceases. The rate and nature of growth of the cotyledon will naturally vary with environmental conditions; hence variation in the actual length of the infection period is to be expected. The mature

basal sheath of the cotyledon thus protects the embryonic region of the younger leaves from infection. That portion of the first leaf which emerges from the cotyledon is susceptible to infection for some little time after emergence, but since it ordinarily is not actually in contact with infested soil, this fact is probably not of practical significance.

TABLE II.—*Relation of stage of development of the onion seedling to infection by Urocystis cepulae*

| Pot No. | Method of exposure to inoculation. | Interval between sowing and inoculation. | Length of cotyledon at time of inoculation | Condition of first leaf at time of inoculation. | Interval between inoculation and final examination. | Total number of plants. | Percentage smutted. |
|---|---|---|---|---|---|---|---|
| | | *Days.* | *Inches.* | | *Days.* | | |
| 1-3 | Uninoculated......... | .. | ... | .......... | ..... | 100+ | 0 |
| 4 | Infected soil around base of cotyledon. | 13 | 1 | Not out .... | 27 | 24 | 75 |
| 5 | Do.... | 16 | 1½ | ...do..... | 24 | 34 | 35 |
| 6 | Do.... | 19 | 2 to 2¼ | ...do..... | 24 | 41 | 8 |
| 7 | Do.... | 24 | 2 to 2¼ | Just out. .. | 24 | 19 | 0 |
| 8 | Do.... | 31 | 2 to 2¼ | ½ to 1½ inches above axis. | 24 | 36 | 0 |
| 9 | Cotyledons removed and infected soil placed around base of first leaf. | 31 | 2 to 2¼ | ..do .... | 24 | 15 | 60 |
| 10 | One-inch layer of infected soil placed on surface of old soil so as to cover lower inch of aerial portions of plants. | 40 | 2 to 2½ | ½ to 4 inches above axis. | 24 | 36 | 6 |
| 11 | Same as No. 10, except that pot was transferred to a temperature of 25° to 28° C. | 40 | 2 to 2½ | ..do...... | 24 | 46 | 28 |

## RELATION OF SOIL MOISTURE TO INFECTION

Since there are these well-defined limits to the time of smut infection, the possibility becomes clearly evident that variable environmental factors during this limited period may exercise a controlling influence on the occurrence of the disease. As already stated, the problem seems to resolve itself primarily into the question of the relations of soil temperature and soil moisture to infection. The results of several workers upon the grain smuts, as summarized by Jones (*5*), have shown that soil temperature may influence infection. Hungerford and Wade (*4*) have published evidence that high soil moisture, also, may favor infection of wheat by the smut fungus *Tilletia tritici* (Beij.) Wint. Variations in the moisture content of the surface layers of soil are likely to be wide, especially during the late summer planting season in the southern States when high temperatures and low humidity may cause rapid desiccation.

An experiment was therefore carried out in which onion seedlings were grown in smut-infested soil in pots which were held at different degrees of soil moisture. Galvanized iron pots 5 inches in diameter and 4 inches deep were used for these trials. Greenhouse sandy loam soil was used and its water-holding capacity was determined in advance by the two standard methods recommended by soil physicists [1]—that is, by means of the 10-inch cylinder and the 1-cm. cup. The soils were brought to the desired low and medium water contents before they were placed in the culture pots; and in those cases where the desired moisture approached the water-retaining capacity, the water content was finally adjusted after the soil was potted. Although these methods failed to secure exact uniformity in the physical compactness of the soils in the several series, they were considered satisfactory as to initial moisture conditions. The pots were weighed daily during the progress of the experiment, and water was added to replace the losses. Since it was realized that the surface layer of soil would change in moisture content through evaporation more rapidly than the lower layers, an effort was made to reduce this surface evaporation so far as practicable. To this end, tar paper covers were used until the seedlings came above ground, when glass covers were substituted for a few days, and finally mineral wool was packed on the surface about the seedlings to reduce evaporation. Absolutely uniform moisture throughout the pot could not be maintained even by this method, and the upper layers of soil unavoidably assumed a somewhat lower water content than the average for the pot. Therefore, at the end of the experiment, moisture determinations were made of the upper inch of soil, since this was the important part from the standpoint of smut infection.

The soil was inoculated at the outset by the introduction of spores from diseased leaves and scales. The data from this experiment are given in Table III. Good germination took place within the range of 10 to 15 per cent moisture content (45 to 70 per cent of the moisture-holding capacity). A high percentage of infection also occurred within this range. Above 15 per cent there was some decrease in germination together with a gradual reduction in infection. At the extreme, however, where germination of seed was practically eliminated (pot 1), one of the two plants surviving became infected. It is evident from these data that a good percentage of infection may be expected at a soil moisture content up to the limit where good germination and growth of the host plant occur. There was a gradual reduction of infection below 5 per cent (23 per cent of the moisture-holding capacity), but this was not sufficient to insure good germination and support good growth. It may be concluded, therefore, that soil moisture does not function as a factor limiting infection with onion smut within the limits at either extreme where good germination and growth of the host occur.

[1] We are especially indebted to Prof. E. Truog, of the Department of Soils of the University of Wisconsin for advice in connection with this work.

TABLE III.—*Relation of soil moisture to infection by Urocystis cepulae*

| Pot No. | Original moisture content (percentage of dry weight).[a] | Moisture content of surface layer at end of experiment. | Number of seeds planted. | Total number of plants. | Percentage of plants smutted. |
|---|---|---|---|---|---|
| 1 | 19.6 | 27.1 | 100 | 2 | 50 |
| 2 | 17.1 | 24.7 | 100 | 8 | 0 |
| 3 | 16.3 | 23.7 | 100 | 52 | 42 |
| 4 | 18.2 | 23.4 | 100 | 48 | 71 |
| 5 | 20.0 | 14.0 | 100 | 50 | 90 |
| 6 | 15.0 | 12.4 | 100 | 60 | 97 |
| 7 | 15.0 | 11.6 | 100 | 66 | 98 |
| 8 | 10.0 | 9.3 | 100 | 66 | 98 |
| 9 | 10.0 | 9.0 | 100 | 71 | 85 |
| 10 | 10.7 | 2.4 | 100 | 66 | 94 |
| 11 | 5.0 | 2.5 | 100 | 32 | 59 |
| 12 | 8.1 | 1.4 | 100 | 66 | 50 |
| 13 | 7.5 | 3.4 | 100 | 63 | 19 |
| 14 | 6.6 | 1.3 | 100 | 37 | 35 |

[a] Water-holding capacity, as determined by 10-inch cylinders, was 22.3 per cent; as determined by the 1-cm cup, it was 27.8 per cent.
The calculated wilting coefficient of the plants was 2.3 per cent.

## TEMPERATURE RELATIONS

In calling attention to the importance of soil temperature as a factor in the development of certain plant diseases, Jones (5) points out that several investigators have stressed its bearing upon infection in the case of stinking smut of wheat, *Tilletia tritici* (Beij.) Wint., and of the oat smuts, *Ustilago avenae* (Pers.) Jens. and *Ustilago levis* (K. and S.) Mag. Heald and Woolman (3) showed that the amount of infection with the stinking smut of wheat was reduced as the mean soil temperature rose above 65° F. (18.3° C.) or fell below 40° F. (4.4° C.). Humphrey (4) states, for the same disease, that soil temperatues of 0° to 5° C. and above 22° C. are decidedly unfavorable to infection.

In studying the relations of soil temperature to the developemnt of a parasitic disease, consideration must be given to the possible influence of such temperature upon the host and the parasite independently. This may enable one to analyze with more confidence the effects when host and parasite are subjected simultaneously to the experimental condition. This has been done as far as practicable in connection with the present work.

Unfortunately, germination of the fungus spores under artificial conditions has been so scanty that the effect of temperature upon the fungus has been necessarily limited to inoculation experiments with infested soil. However, the disease is produced so readily and consistently by the latter method that a very accurate index to the development of the fungus can be secured by varying the condition of the infested soil during the germination and early growth of the onion seedling.

The soil-temperature experiments were all carried out in the greenhouse at Madison, Wis., during the winter months. The apparatus in use

in the Department of Plant Pathology, University of Wisconsin, for the control of soil temperatures has been described by Jones (5). Briefly, it consists of a series of water baths held at constant or nearly constant temperatures in which the glass or galvanized-iron culture pots are inserted.[1]

For these experiments galvanized-iron cylindrical pots 5 inches in diameter and 8 inches in depth were used. In order to overcome the influence of the air temperature upon the upper layer of soil, the surface of the latter was kept at ½ to 1 inch below the level of the water. Tar-paper covers were placed over the pots until the seedlings came above ground; these covers were then removed, and a layer of mineral wool was placed on the surface of the soil. By this procedure the temperature of the upper inch of soil was kept reasonably close to that of the deeper portions—that is, approximately that of the water in the tank. In order to follow any minor variations, readings were taken three times daily from thermometers inserted 1 inch below the surface of the soil. At the beginning of the experiments the moisture content of the soil was adjusted to two-thirds of the water-holding capacity. The pots were thereafter weighed at intervals of one to three days, depending upon the rate of water loss, and the moisture content was readjusted accordingly, either by adding water directly to the surface or by introducing it at the bottom of the pot through a glass tube. Obviously this method did not secure uniform distribution of moisture throughout the pot, and unavoidably the content of the upper layer of soil was somewhat lower than the average for the whole pot. It is believed that this variation had little if any influence, however, since other experiments, described earlier in this paper, showed that infection is quite uniform over a much wider range of soil moisture than here occurred. The seed was planted at a depth of 1 inch. Since the chlorophyll in the tops in some cases obscured the smut lesions, the plants were placed in alcohol acidified with acetic acid until thoroughly bleached before final examination for the disease was made.

## EFFECT OF SOIL TEMPERATURE UPON THE DEVELOPMENT OF THE HOST

Experimental studies to determine the relation of soil temperature to the rate and character of seed germination and seedling development were carried on in conjunction with those relating to infection, of which the results will be presented in the next section. It will be simpler, however, to discuss these two aspects of the problem separately, taking up first the relations of temperature to host development.

EXPERIMENT I.—Seven pots of sterilized greenhouse loam soil were uniformly planted with 50 Red Globe onion seeds in each pot. One pot was then held at each of the following temperatures: 10° to 14°, 16.5°

[1] Since this description was published, numerous improvements have been made from time to time by members of the Department. As now in use these are termed the "Wisconsin soil temperature tanks."

to 18°, 19° to 22°, 24° to 26°, 27° to 29°, 30° to 31°, 35° C. The moisture content of the soil was held at two-thirds the water-holding capacity (22 per cent of dry weight). The air temperature of the greenhouse was kept at about 15° with a rise to 20° during the middle of the day. These conditions as to soil moisture and air temperature were such as had proved favorable for both host and parasite development in the earlier trials. The first seedlings to appear above ground were those at 27° to 29°, those at 24° to 26° came up shortly afterward, then those at 19° to 22°. Good growth took place at these three temperatures, but germination was very slow at lower temperatures. At the highest temperature, 35°, a few seeds germinated, but growth was very slight. The plants were all removed and the roots washed out on January 7, 1920, 29 days after the seed was sown. The data given in Table IV summarize the condition of the plants at this date. It will be seen that at this early stage in the development of the plants there was a tendency for best root development at about 21° or below, while the best development of tops took place at this point or above.

TABLE IV.—*Development of onion seedlings in sterilized greenhouse soil held at 22 per cent of the dry weight or two-thirds the moisture-holding capacity, and at different soil temperatures. Data on January 7, 1920, 29 days after sowing*

| Soil temperature. | Number of seeds planted. | Total number of plants. | Percentage with 2 roots. | Percentage with 3 roots. | Percentage with first leaf. | Average dry weight of tops per plant. | Average dry weight of roots per plant. | Average total dry weight per plant. |
|---|---|---|---|---|---|---|---|---|
| °*C* | | | | | | *Gm.* | *Gm.* | *Gm.* |
| 10 to 14 | 50 | 41 | 22 | 0 | 17 | 0.00224 | 0.00046 | 0.0027 |
| 16.5 to 18 | 50 | 30 | 17 | 3 | 70 | .00293 | .00023 | .0031 |
| 19 to 22 | 50 | 32 | 56 | 25 | 100 | .00396 | .00043 | .0044 |
| 24 to 26 | 50 | 28 | 32 | 0 | 82 | .00307 | .00028 | .0033 |
| 27 to 29 | 50 | [a] 14 | 50 | 0 | 86 | .00285 | .00014 | .0030 |
| 30 to 31 | 50 | [a] 8 | 13 | 0 | 63 | .00212 | ......... | .0021 |

[a] The reduced stand at 27° to 29° and 30° to 31° C. was due to damping-off fungi.

EXPERIMENT II.—The experiment was repeated, with some modifications, starting April 10, 1920. The Red Globe and Yellow Bermuda varieties were used. Two pots of each variety were kept at each of the following soil temperatures: 14°, 20°, 25°, 28°, 30° C. The air temperature ran slightly higher (20° to 30°) during the middle of the day and dropped to about 15° for the most of the night. Both the rate and the percentage of germination were noted, and the data are recorded in Table V. In both varieties the most rapid germination took place at 25°, although the rate was only slightly less at 20°, 28°, and 30°. At 14° the seedlings were distinctly slower in starting off. The plants from one pot of each series were removed on the thirteenth day. The dry weights of the tops and roots as given in Table V were so small at this age that comparison on this basis does not have any great value. The tendency for rapid development of tops as compared with roots at 20° or above is, however, shown very strikingly in Plate 25. The plants in the remaining

pots were removed on the thirtieth day. The relative dry weights of roots and tops then secured are shown in Table V and those of the Red Globe are graphed in figure 1.

While the temperature relations of the two varieties were alike in their main features, there was an interesting minor difference, possibly indicative of the better adaptation of the Red Globe for northern culture and of the Yellow Bermuda for southern. In both cases with these onions, as *indeed holds generally in our experiments with other plants, the best*

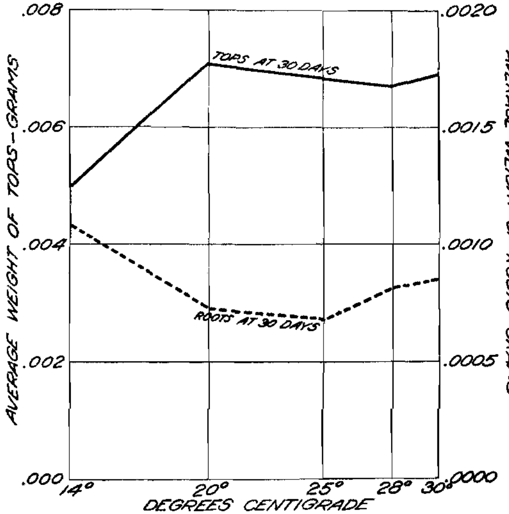


FIG. 1.—Relative developments of dry weight in tops and in roots of Red Globe onion as shown at end of 30 days' growth in a series of culture pots kept at the several soil temperatures indicated, with all other factors, including air temperature, alike for all. Note that the best root development occurs at the low temperatures (12° to 15° C.) whereas the tops are forced more strongly at higher temperatures (20° to 25°).

root development occurs at relatively lower temperatures (12° to 20° C.) while best top development occurs at higher temperatures (20° to 25°). When the varieties are compared, it is seen that with the Globe both roots and tops grew relatively better at somewhat lower temperatures than did those of the Bermuda. Thus the data at 30 days show the maximum root development of the Globe at 14° while that of the Bermuda was at 20°; with tops the maximum was at 20° for the Globe and at 25° for the Bermuda.

TABLE V.—*Rate of onion seed germination and of subsequent seedling development at various soil temperatures with a uniform air temperature of 15° to 20° C.*

| Variety. | Soil temperature. | Percentage of germination. | | | | 13-day-old plants. | | | | 30-day-old plants. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Seventh day. | Ninth day. | Eleventh day | Thirteenth day. | Number of plants | Average weight of tops. | Average weight of roots | Average stage of development | Number of plants | Average weight of tops. | Average weight of roots | Average stage of development. |
| | °C. | | | | | | Gm. | Gm. | | | Gm. | Gm. | |
| Red Globe | 14 | 0 | 0 | 7 | 59 | 58 | 0.00126 | 0.00026 | Cotyledon, first root | 60 | 0.00499 | 0.00108 | First leaf; third root. |
| | 20 | 25 | 65 | 73 | 73 | 72 | .00175 | .00037 | do | 67 | .00709 | .00073 | Second leaf; first and second roots. |
| | 25 | 63 | 72 | 74 | 74 | 56 | .00186 | .00033 | do | 44 | .00684 | .00068 | Second leaf; first root. |
| | 28 | 52 | 66 | 68 | 70 | 60 | .00166 | .00029 | do | 49 | .00672 | .00081 | Second leaf; first root. |
| | 30 | 48 | 65 | 67 | 69 | 57 | .00168 | .00030 | do | 45 | .00691 | .00085 | Second leaf; first root. |
| Yellow Bermuda | 14 | 0 | 0 | 72 | 56 | 54 | .00133 | .00040 | do | 46 | .00717 | .00108 | First leaf; third root. |
| | 20 | 37 | 63 | 66 | 68 | 45 | .00180 | .00040 | do | 68 | .00766 | .00122 | Second and third leaf; second and third roots. |
| | 25 | 54 | 64 | 65 | 68 | 49 | .00196 | .00044 | do | 41 | .00896 | .00119 | Second leaf; first root. |
| | 28 | 43 | 53 | 59 | 56 | 32 | .00175 | .00037 | do | 33 | .00569 | .00084 | Second leaf; first root. |
| | 30 | 49 | 58 | 59 | 61 | 49 | .00182 | .00040 | do | 45 | .00597 | .00075 | First and second leaves; first root. |

## EFFECT OF SOIL TEMPERATURE UPON INFECTION

At present the chief interest in these data focuses upon the question of any possible bearing of the rate of development of the host plant at different temperatures upon predisposition to, or escape from, smut infection, recalling that such infection is practically limited to the seedling stage before the maturity of the cotyledon. It has just been noted that the promptest seed germination and most rapid growth of tops during this early seedling stage occur at fairly high temperature, 20° to 25° C., with a rather pronounced drop in rate of aerial growth at temperatures below 20°. It is to be expected, therefore, that in the northern onion-growing sections where the seed is planted in early spring there will be a rather tardy germination and slow early development of tops, the growth energies during the seedling stage being directed under this climatic environment to a relatively stronger development of the root system. In the South where the seed is planted in the comparatively warm period of early autumn, we should expect a more rapid top growth at the outset, with correlated strength of root development coming later in the autumn as the soil becomes gradually cooler.

The naturally infested soil was secured near Racine, Wis., from a badly diseased field of sandy loam rich in organic matter. The soil which was artifically inoculated consisted of a greenhouse mixture of loam and sand to which were added fresh spores from smutty onion leaves. In order to test the efficacy of this method of soil inoculation a preliminary planting of onion seed was made in advance of the final experiments. This gave a high percentage of smut infection, showing that the method of introducing the inoculum was satisfactory. Several early trials indicated that below 25° C. soil temperature variations have little effect on the relative amount of infection. The results of two such experiments, nearly covering the range of onion seed germination, are given in Table VI (experiments 1 and 2). It is evident from these figures that abundant infection occurred between 10° and 25°, both with naturally infested and with artificially inoculated soil. The number of pustules per plant as shown in Plate 25 proves that the fungus was very active even at low temperatures. Above 25° infection is reduced very rapidly, as indicated by both the percentage of infected plants and the number of pustules per plant.

In order to determine more closely the point at which infection is inhibited four more serial experiments were conducted (experiments 3 to 6) in which the temperature was kept as constant as possible at 2-degree intervals between 25° and 31° C. The results given in Tables VII and VIII, and illustrated in Plates 26 and 27, show that abundant infection took place at 25° to 26°, while it was greatly reduced at 27° to 28° and completely inhibited at 29° or above. The infected seedlings from experiment 5 show the great reduction in the amount of disease per

plant at 27° to 28° as compared with 25° to 26°. It is interesting to note that infection was reduced more abruptly at 27° to 28° in the artificially inoculated soil than in that naturally infested. This may be due to the age of the inoculum, a smaller percentage of the spores being functional in the former soil, or perhaps to the presence of a smaller amount of inoculum.

TABLE VI.—*Relation of soil temperature to infection of onion seedlings by Urocystis cepulae*

| Experiment 1. | | | Experiment 2. | | |
|---|---|---|---|---|---|
| Naturally infested soil. Begun May 3, 1919, completed May 30, 1919. Records not kept as to soil moisture nor as to air conditions. | | | Artificially inoculated soil. Begun Dec. 10, 1919; completed Jan. 6, 1920. Soil moisture held at 22 per cent or two-thirds the water-holding capacity. Air temperature 13° to 25° C., relative humidity 45 to 75 per cent. | | |
| Soil temperature. | Total number of plants. | Smutted plants. | Soil temperature. | Total number of plants. | Smutted plants. |
| °C. | | Per cent. | °C. | | Per cent. |
| 10 to 13 | 25 | 72 | 10 to 14 | 64 | 98 |
| 18 to 20 | 5[a] | 80 | 16.5 to 18 | 49 | 98 |
| 22 to 24 | 6[a] | 100 | 19 to 22 | 63 | 100 |
| 25 to 30 | 47 | 15 | 23 to 26 | 56 | 93 |
| 28 to 34 | 25 | 0 | 27 to 29 | 52 | 8 |
| | | | 29 to 31 | 36 | 0 |

[a] Stand reduced by damping-off fungi.

TABLE VII.—*Relation of soil temperature to infection of onion seedlings by Urocystis cepulae*

| Experiment 3. | | | Experiment 4. | | |
|---|---|---|---|---|---|
| Naturally infested soil. Begun Dec. 20, 1919; completed Jan. 10, 1920. Soil moisture held at 25 per cent or two-thirds the water-holding capacity. Air temperature, 13° to 25° C.; relative humidity, 45 to 75 per cent. | | | Artificially inoculated soil. Begun Dec. 18, 1919; completed Jan. 12, 1920. Soil moisture held at 13 per cent or two-thirds the water-holding capacity. Air temperature, 13° to 25° C.; relative humidity, 45 to 75 per cent. | | |
| Soil temperature. | Total number of plants. | Smutted plants. | Soil temperature. | Total number of plants. | Smutted plants. |
| °C. | | Per cent. | °C. | | Per cent. |
| 19 to 22 | 44 | 93 | | | |
| 23 to 26 | 131 | 96 | 23 to 26 | 50 | 98 |
| 26 to 27 | 100 | 57 | 26 to 28 | 58 | 12 |
| 27 to 28 | 86 | 12 | 29 to 31 | 40 | 0 |

TABLE VIII.—*Relation of soil temperature to infection of onion seedlings by Urocystis cepulae*

| Experiment 5. | | | Experiment 6. | | |
|---|---|---|---|---|---|
| Naturally infested soil. Begun Jan. 16, 1920; completed Feb. 10, 1920. Soil moisture held at 25 per cent or two-thirds the water-holding capacity. Air temperature, 13° to 25° C.; relative humidity, 40 to 80 per cent.. | | | Artificially inoculated soil. Begun Jan. 16, 1920; completed Feb. 10, 1920. Soil moisture held at 25 per cent or two-thirds the water-holding capacity. Air temperature, 13° to 25° C.; relative humidity, 40 to 80 per cent. | | |
| Soil temperature. | Total number of plants. | Smutted plants. | Soil temperature. | Total number of plants. | Smutted plants. |
| °C. | | Per cent. | °C. | | Per cent. |
| 25 to 26 | 89 | 100 | 25 to 26 | 106 | 90 |
| 27 to 28 | 103 | 47 | 27 to 28 | 98 | 8 |
| 29 to 30.5 | 77 | 0 | 29 to 30.5 | 47 | 0 |
| 30 to 32 | 43 | 0 | 30 to 32 | 30 | 0 |

It may be concluded from the foregoing experiments that a high percentage of infection may be expected up to 25° C., above which there is a rather abrupt reduction, leading to complete inhibition at 29°. There appears to be no lower limit of temperature for infection within the range where onion seeds will germinate and normal growth occur.

After it was clearly shown that no infection would take place at 29° C., the question arose as to how long seedlings must grow at this temperature to become entirely immune. It has been shown that at moderate temperatures the plant becomes immune in about 20 days, or at about the time when the cotyledon has reached its maximum growth. To determine whether or not this condition is altered when the plants are grown at 29°., several pots each of the naturally and the artificially inoculated soil were started off at this high soil temperature. Pots were then transferred from time to time to a lower temperature favorable for infection (15° to 20°) where they were held for about three weeks before they were examined for signs of the disease. The results of these experiments are summarized in Table IX.

It is quite evident that the amount of infection was markedly reduced by an exposure of 15 to 18 days at 29° C. Complete inhibition of infection by even more protracted exposure to this high temperature was not attained. However, where infection did occur there was usually not more than one lesion per plant, which in the majority of cases was so situated that subsequent infection of newly forming leaves would be impossible. It is thus quite certain that where seedlings develop at about 29° for the first 20 days the amount of damage from smut will be negligible, especially in an area where the amount of inoculum is slight.

TABLE IX.—*Effect of different soil temperatures upon onion smut infection. In all cases except the fifth, tenth, and eleventh, the pots were held for the stated period at 29° C., where infection was inhibited, then transferred to 15° to 20°, a temperature favorable for infection. In the fifth and tenth, where the continuous temperature was high, note that practically no smut developed; in the eleventh, where the soil temperature was continuously low, note that practically all the plants were smutted; in the remaining series, where the plants were transferred from the higher temperature (29°) to the lower (15° to 20°) after varying periods, note that long exposures at the higher temperature tended to reduce the amount of infection.*

| Pot No. | Type of soil inoculation. | Length of exposure to 29° C. | Size of plants at time of removal to low temperature. | Extent of infection at end of experiment. | | |
|---|---|---|---|---|---|---|
| | | | | Total number of plants. | Percentage diseased. | Extent of infection. |
| | | *Days.* | | | | |
| 1 | Artificially inoculated soil. | 11 | Cotyledons 1 inch long. | 16 | 94 | 63 per cent diseased first leaf. |
| 2 | | 15 | Cotyledons 2 to 2½ inches long. | 17 | 41 | 12 per cent diseased first leaf. |
| 3 | | 18 | .....do........... | 12 | 25 | 25 per cent diseased first leaf. |
| 4 | | 28 | First leaves 1+ inches high. | 9 | 11 | 11 per cent diseased first leaf. |
| 5 | | 35 | Continuous exposure at 29°. | 6 | 0 | |
| 6 | Naturally infested soil. | 12 | Cotyledons 1½ to 2 inches long. | 29 | 48 | 14 per cent diseased first leaf. |
| 7 | | 16 | Cotyledons 2½ to 3½ inches long. | 40 | 13 | 3 per cent diseased first leaf. |
| 8 | | 23 | First leaves out in about one-half plants. | 24 | 8 | 8 per cent diseased first leaf. |
| 9 | | 27 | First leaves out in most plants. | 16 | 13 | 6 per cent diseased first leaf. |
| 10 | | 38 | Continuous at 29°. | 34 | 3 | 0 per cent diseased first leaf. |
| 11 | | 0 | Continuous at 15° to 20°. | 76 | 99 | Most of these plants died in cotyledon stage. |

It is interesting to note also in this connection that continued exposure of onion roots to a temperature of 29° C. led to the gradual slowing up of growth. With the transfer of the pots to the lower temperature (15° to 20°) both root and top development were greatly stimulated. In attempting to correlate these results one must keep in mind the fact that in nature the temperature conditions under which the onions develop are much different from those in the experimental pots. Whereas in the pots the soil temperature is uniform throughout their depth, in the natural soil there is a gradual decrease in temperature at progressively greater depths. The temperature of the upper inch runs extremely high during the summer months because of direct exposure to the sun's rays, and this is the area critical for infection by onion smut. The young roots, on the other hand, as they develop progressively reach strata of lower temperature, which are more favorable for their growth.

## EFFECT OF HIGH AIR TEMPERATURE UPON THE DEVELOPMENT OF SMUT

The experiments reported above in which the soil temperature was varied were carried on at an air temperature of 15° to 20° C. The latter is considerably lower than the air temperature which prevails in southern onion sections at the time when young seedlings are starting off. This is shown for one section (Laredo, Tex.) in Table XIII, where the mean air temperature is about 30° during most of the onion-planting time. The question arose as to what effect these high air temperatures might have upon the development of smut in the aerial portions of the plant. Five clay pots of onions in naturally infested soil were started off in a greenhouse running at about 25°. Previous observations had shown that the pustules become evident in the cotyledons on about the twefth day under these conditions. Accordingly the plants were allowed to grow at this temperature for nine days, at which time a few lesions were barely visible. In order to prove that good infection had already taken place, 10 plants were removed from infested soil and washed thoroughly in running water to remove any external inoculum, after which they were transplanted to clean soil. Within two days lesions were distinctly visible in these plants, and smut developed in 8 out of the 10.

As a control on this method of removing the external inoculum 11 plants grown in clean soil were moistened and covered thoroughly with infested soil. They were then washed in running water and transplanted to clean soil. No smut developed. On the ninth day after sowing, 4 of the 5 pots were removed to a greenhouse running at 30° to 33° C., one being allowed to remain at 25°. One pot was then transferred from the higher temperature back to 25° at the end of each the second, fourth, ninth, and fourteenth days. This exposure to the higher temperature resulted in a stimulation of host plant growth for a few days. When the plants were allowed to remain at this high temperature, however, for three weeks distinct stunting became evident, while more prolonged exposure resulted in death. The plants so transferred were allowed to continue growth at 25° for three weeks or more, when they were examined for the presence of smut. The final results are given in Table X. It was evident that the gradual elimination of smut which took place was proportional to the length of exposure to the higher temperature (29°). After 14 days of exposure only small lesions developed on 16 per cent of the onion plants, although presumably 80 per cent or more of these plants were originally infected while they were growing at the lower temperature. This experiment was repeated several times with practically the same results, namely, that exposure of plants bearing incipient infections to a temperature of 30° to 33° for 12 to 15 days almost entirely checked further development of the parasite.

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TABLE X.—*Effect of high temperature, following infection, in inhibiting the further development of smut. The plants were from a series of pot cultures started at 25° C. and held there until incipient infection had occurred, then transferred for the period indicated to a high temperature, 30° to 33°, and finally brought back to the original 25°.*

| Length of exposure to temperature of 30° to 33°. | Number of plants. | Percentage smutted. |
|---|---|---|
| None (25° throughout) | 34 | 94 |
| 2 days | 29 | 45 |
| 4 days | 30 | 37 |
| 9 days | 40 | 33 |
| 14 days | 36 | 17 |

It is to be noted that in the experiments just reviewed potted plants were used. The entire pot, thus including roots and tops of the experimental plants, was exposed to the stated temperature condition.

The question then arose whether the results secured were due entirely to the effect of high air temperature upon the fungus or to an indirect effect of the changed conditions upon the metabolism of the host. In order to throw some light upon this point two experiments were conducted in which seedlings were grown in infected soil at three constant soil temperatures and each of two air temperatures, 25° and 30° to 33° C. The results (Table XI) at the lower air temperature (25°) coincided closely with those previously secured at air temperatures of 13° to 25°, inasmuch as abundant infection occurred when the soil temperature was 25°, while complete inhibition was attained at 30°. It is, however, significant to note, in comparison with the results in Table X, that, with the soil temperature held at 20° or 24°, the 30° to 33° air temperature did not greatly check the development of the disease. It appears, then, that roots as well as tops must be exposed to the inhibitive higher temperature, 30° to 33°, in order fully to check the parasite after incipient infection has taken place. This suggests that the inhibitory effect may be due in part at least to the influence of the environmental conditions upon the metabolism of the host and not entirely to a direct effect upon the fungus itself.

TABLE XI.—*Effect of different combinations of soil and air temperature upon onion smut infection*

| Air temperature. | Soil temperature. | Experiment 1. | | Experiment 2. | |
|---|---|---|---|---|---|
| | | Number of plants. | Percentage smutted. | Number of plants. | Percentage smutted. |
| °C. | °C. | | | | |
| 25 | 20 | 23 | 100 | 85 | 95 |
| | 25 | 68 | 97 | 49 | 92 |
| | 30 | 42 | 0 | 22 | 0 |
| 30 to 33 | 20 | [a] 9 | 89 | 41 | 46 |
| | 24 | 73 | 86 | 50 | 60 |
| | 30 | 24 | 0 | 30 | 0 |

[a] Stand reduced by damping-off fungi.

## EFFECT OF MODERATELY HIGH TEMPERATURES UPON SYSTEMIC INVASION OF THE PLANT

Thaxter (*10, p. 134*) observed that in some instances the smut fungus may infect and develop in the cotyledon without invading the first leaf, with the result that the plant eventually outgrows the disease. Observations lead us to believe that this may vary with different temperatures. It has been pointed out that at a temperature of about 25° C. the most rapid top growth of the onion seedling occurs, while at temperatures below 20° the top growth is much retarded. Two pots of infested soil were sown with onion seed and placed in greenhouses, one at 24° to 28°, with a maximum of about 36°, for one or two hours on sunny days, the other at 15° to 20° A high percentage of cotyledon infection occurred in both pots. After 31 days 24 out of 29 plants at the high temperature were infected, but the pustules were all confined to the cotyledons and no infection of first leaves had developed, although the plants were now in the second leaf stage. At the low temperature, on the other hand, of approximately the same number of plants, only 9 had survived on the thirty-seventh day, and 8 of these showed infection in the second leaves. It appears, then, that rapid growth of tops at about 25° may result in a large percentage of plants outgrowing the disease after the cotyledons become infected. The results of successive field plantings, discussed in the next paragraph, seem to confirm this judgment. The importance of the practical bearings of this matter are such as to justify further critical attention.

## EFFECT OF SUCCESSIVE PLANTINGS THROUGHOUT THE GROWING SEASON UPON INFECTION

The laboratory experiments described early in this paper have shown that onion smut infection is greatly reduced where a constant soil temperature of 27.5° C. is maintained during the susceptible period of the plant's growth, while a temperature of 29° thus applied completely inhibits infection. Moreover, as explained in the last paragraph, when plants are growing in infested soil with temperature of air and soil held at about 25°, although a high percentage of cotyledon infection may occur, there is a greater tendency than at lower temperatures for the plants to outgrow the disease, owing to the rapid growth of tops. These results combined to justify the expectation that successive field plantings of onion seed throughout the growing season might show considerable variations in the percentage of smut infection. In the onion field the soil temperature usually varies widely during 24 hours, often reaching a maximum considerably above 29° during the day and descending to a minimum much below this at night. Under Wisconsin conditions the daily mean temperature gradually rises during the spring and early summer months and falls during the latter part of the growing

season. It seemed possible, therefore, that by making successive plantings a period might be found for this latitude when the mean soil temperature is sufficiently high to materially check or completely inhibit onion smut infection.

In order to test this out, a series of plantings at intervals of from 8 to 14 days was begun on June 18, 1920, at Madison, Wis. Onion seed was sown in smut-free soil in an open trench about 1 inch deep and was then covered with naturally infested soil similar to that used in certain of the laboratory experiments. Two varieties, Red Globe and Yellow Bermuda, were used, one 10-foot row of each variety being put in at

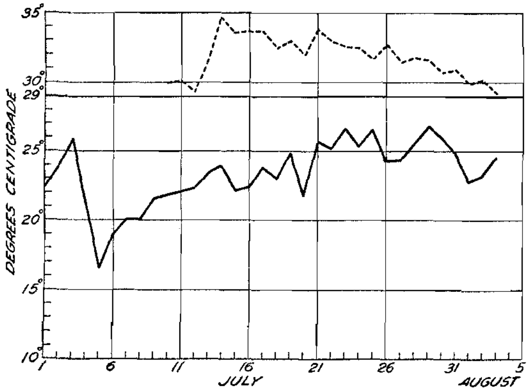


Fig. 2.—Graph showing the daily mean soil temperature at a depth of 1 to 2 inches as it occurred in the "successive planting" plots. Since the weather continued rather cool, one bed was covered with glass to insure a higher temperature. The temperature of the uncovered bed is shown by the solid line, the temperature of the glass-covered bed by the broken line. For further details see Table XII and and accompanying text.

each planting. Temperatures of the soil at a depth of 1 to 2 inches were obtained by means of a self-recording thermograph. The hourly mean temperature for each day was then secured by adding temperatures as recorded for each hour and dividing the sum by 24. These computations are represented graphically in figure 2. Since the weather in July was unusually cool, a higher mean soil temperature was secured for some of the plots by covering them with an ordinary glass cold frame. Inasmuch as the dry weather and high temperature would cause a rapid desiccation of the surface layer of soil, the plots were watered thoroughly on alternate days or oftener during the early growth of the plants. The

data collected from this field plot are tabulated in Table XII. Observations were made by pulling plants at several points in each plot, and examining for smut lesions after the chlorophyll had been removed by means of alcohol and acetic acid. The first observation was made on the twenty-first to the twenty-third day after planting. Subsequent observations were made as indicated in the table.

TABLE XII.—*Development of onion smut in successive plantings in the field at Madison, Wis., 1920*

| Date of planting, 1920. | Variety. | Treatment. | First observation, 21 to 23 days after planting. | | Second observation, 29 to 31 days after planting. | | Third observation, 44 to 65 days after planting. | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Number of plants examined. | Percentage smutted. | Number of plants examined. | Percentage smutted. | Time after planting (days). | Number of plants examined. | Percentage smutted.[g] |
| June 18 | Red Globe | Uncovered | 108 | 53 | [b] 50 | [c] 40 | 52 | 31 | 3 |
| June 26 | Red Globe | ...do | 62 | 73 | 51 | [d] 76 | 44 | 51 | 39 |
| | Yellow Bermuda. | ...do | 45 | 49 | 42 | [e] 86 | ...... | ...... | .... |
| July 10 | Red Globe | ..do | 41 | 10 | 47 | [f] 23 | 65 | 61 | 0 |
| | Yellow Bermuda. | ...do | 20 | 10 | 28 | [f] 7 | 65 | 24 | 0 |
| | Red Globe | Covered | 52 | 0 | 33 | 0 | 65 | 9 | 0 |
| July 19 | Red Globe | Uncovered | 40 | 13 | ...... | ...... | 53 | 135 | 11 |
| | Yellow Bermuda. | ..do | 35 | 14 | ...... | ...... | 53 | 39 | 13 |
| | Red Globe | Covered [a] | 46 | 0 | ..... | ...... | 53 | 91 | 9 |
| | Yellow Bermuda. | Covered [a] | 26 | 0 | . | ..... | 53 | 45 | 24 |

[a] Covered for 15 days only.
[b] Observation 39 days after planting.
[c] Extent of infection: Systemic, 26 per cent, confined to dead cotyledon, 14 per cent.
[d] Extent of infection: Systemic, 10 per cent; confined to dead cotyledon, 66 per cent.
[e] Extent of infection Systemic, 5 per cent; confined to dead cotyledon, 81 per cent.
[f] Extent of infection: All cotyledon infections
[g] Extent of infection. All systemic infections.

An analysis of the data secured can be made by referring to Table XII and figure 2. It will be seen that the soil temperature mean gradually rose until July 23 to 29, after which there was a gradual drop. At no time did the mean in the uncov red plot reach the inhibiting temperature (29° C.), but it closely approached this point during the warmest portion of the season. In the covered plot, however, the mean remained above 29° continuously until the cover was removed on August 3. The two important points to be considered in the respective plantings were (1) the amount of original infection which was determined three to four weeks after planting (see first and second observations in Table XII) and (2) the extent to which the disease either became systemic or was entirely outgrown by the plants during the following four or five weeks (see third observation in Table XII).

Considering first the amount of original infection, it will be seen that a high percentage of disease resulted in all the plantings of June 18 and June 26. The somewhat lower infection in that of June 18 may be explained in part at least by the fact that a smaller quantity of inoculum was used than in subsequent plantings. The next two plantings (July 10 and 19) were so made that the resulting seedlings were exposed during early growth to the maximum soil temperature of the season. By referring to Table XII it will be seen that associated with this higher temperature there was a decided reduction in the amount of infection in even the uncovered plots, while in the covered plots, where the mean temperature remained continuously above 29° C., no infection whatever occurred.

Considering, secondly, the extent to which the disease became systemic or was outgrown, it will be seen that in the planting of June 18 a majority of the infected plants showed systemic invasion at the second observation (thirty-ninth day). In the next planting (June 26), however, by the time of the second observation most of the external signs of the disease were confined to the dead cotyledons. The amount of systemic infection increased somewhat, however, at the third observation (39 per cent).

In the third planting (July 10) it is interesting to note first that the plants in the covered plot remained entirely free from infection. In the uncovered plot, although some cotyledon infection was noted at the second observation, no disease whatever was found at the third observation. This indicates that the time when the temperature was at its highest point the infected plants succeeded best in outgrowing the disease.

The field data secured in the foregoing experiments at Madison are thus in general accord with the experiments performed under controlled conditions. In such controlled experiments the amount of smut infection falls as the soil temperature rises toward 29° C. and is totally inhibited above this temperature. Likewise in the field trials with successive plantings there was a gradual reduction in the amount of infection following the rise in the mean soil temperature, with omplete inhibition of infection where the mean was kept above 29° for two or three weeks after planting. Complete freedom from infection under these Wisconsin field conditions was secured only by growing the plants under artificial conditions in which by covering the plants with glass the temperature was raised several degrees above the normal. It is, however, to be noted that the summer of 1920, when the foregoing results were secured, was somewhat cooler than the average. The weather records of other years indicate that in a hot summer complete inhibition of smut infection would be secured by such summer plantings.

## CORRELATION OF EXPERIMENTAL RESULTS WITH FIELD CONDITIONS OF THE SOUTHERN STATES

These results obtained in both greenhouse and field experiments justify the question as to the part played by soil temperature in determining smut infection in the onion fields of the more southern States. As noted at the beginning of this article, a recent survey of southern onion sections indicates that smut is not prevalent in the southern fields—for example, in Texas—as it is in the northern onion sections. As was earlier explained, it is the practice in these southern fields to plant the onion seed in late summer or early autumn. It is thus quite possible that the mean temperature for the surface inch of soil in southern onion sections is considerably above the maximum for onion smut infection during and immediately following the sowing of seed. According to Mally (*6*), onion seed is sown in the Laredo district of southern Texas as early as August 1, while most of the seed is planted about September 10 to 25. The mean air temperature as recorded at Laredo, Tex., by the United States Weather Bureau for August, September, and October, 1917, is given in Table XIII.

TABLE XIII.—*Mean air temperatures for August, September, and October, 1917, at Laredo, Tex.*[a]

| Day of month. | August. | September. | October. | Day of month. | August. | September. | October. |
|---|---|---|---|---|---|---|---|
| | °F. | °F. | °F. | | °F. | °F. | °F. |
| 1 | 89.5 | 85.5 | 71.0 | 17 | 90.0 | 79.0 | 80.5 |
| 2 | 88.5 | 86.0 | 80.0 | 18 | 90.5 | 83.0 | 81.5 |
| 3 | 88.5 | 86.5 | 80.5 | 19 | 91.0 | 75.5 | 76.0 |
| 4 | 88.5 | 87.0 | 80.5 | 20 | 92.0 | 79.5 | 67.5 |
| 5 | 89.0 | 88.0 | 80.5 | 21 | 90.0 | 79.5 | 62.0 |
| 6 | 88.5 | 87.5 | 78.0 | 22 | 90.0 | 80.0 | 63.5 |
| 7 | 89.0 | 88.0 | 81.0 | 23 | 90.0 | 79.5 | 68.5 |
| 8 | 88.5 | 88.5 | 82.5 | 24 | 91.0 | 79.0 | 61.0 |
| 9 | 89.0 | 89.5 | 64.5 | 25 | 89.5 | 80.5 | 66.5 |
| 10 | 90.5 | 88.0 | 64.5 | 26 | 87.0 | 81.5 | 76.5 |
| 11 | 90.5 | 83.5 | 70.5 | 27 | 89.5 | 81.5 | 74.5 |
| 12 | 88.0 | 85.0 | 73.5 | 28 | 86.5 | 71.5 | 78.5 |
| 13 | 89.5 | 86.5 | 75.0 | 29 | 89.5 | 71.5 | 75.0 |
| 14 | 88.5 | 88.5 | 77.0 | 30 | 84.5 | 71.5 | 50.5 |
| 15 | 90.5 | 83.5 | 76.0 | 31 | 86.0 | ........ | ........ |
| 16 | 88.5 | 83.5 | 80.0 | | | | |

[a] Obtained by averaging the daily maximum and minimum temperatures.

Table XIII shows that the air temperature ranged very high during August and September, the onion-planting period. In this connection it is to be noted, moreover, that the records of Bouyoucos (*1*) in Michigan indicate that surface soil temperatures may considerably exceed air temperatures. Thus, his observations showed that the maximum temperature for the upper quarter inch of all the soils he studied was about 16° C. higher during hot, clear days than that of the air at an elevation

of 4 feet, while the minimum temperature of all the soils used, except peat, was 0.5° to 1.0° C. higher, as a monthly average, than that of the air. Our own observations in Wisconsin are in general accord with these Michigan records. Assuming that the temperature of the surface layer of soil under Texas conditions likewise averages several degrees higher than the air, it is evident that the mean never went below the point where infection is entirely inhibited (29° C., or 84° F.) during August and seldom below it during September. Continuing up to October 8 there were only a few days when the air temperature fell below 27.5° C. (81.5° F.), the point at which our experiments have shown smut infection to be markedly reduced. It seems probable, therefore, that even if onion smut were introduced into this Laredo soil, it would stand small chance of infecting onion seedlings to the extent of establishing the disease as a permanent factor. The data available are not sufficient to justify the attempt at more detailed geographic correlation of onion smut occurrence with the temperature factor. We believe, however, that the conclusion is justified that soil temperature during the early seedling stage must be considered as a limiting factor in determining the occurrence of the disease in any locality. It must be left with local observers to make use of this fact in interpreting conditions as they occur in any particular region.

## SUMMARY

Onion smut was first noted in the Connecticut River Valley in 1869. Since then it has successively appeared and become an economic factor in nearly all of the northern onion-growing sections from New York to Oregon. This has probably resulted from chance introduction of the organism with seed or bottom sets, followed by its accumulation in the soil where continuous cropping with onions is practiced. The disease has not appeared in the southern onion-growing sections of Texas and Louisiana, although they are exposed to similar chance introduction of the parasite and the continuous cropping method is common.

These facts have raised the question as to wherein lies the explanation of the regional limitation of the disease. The southern method of culture, characterized by special seed bed and transplantation of seedlings, does not offer sufficient explanation for the absence of smut. No difference in susceptibility between northern and southern varieties has been found. Is regional limitation explained by differences in environmental factors in the North and the South at the time when the seedling is susceptible to infection, that is, during the first two or three weeks after germination? An analysis of certain of these factors in relation to infection has been the object of the present investigation.

The cotyledon of the onion is susceptible to attack by the smut organism up to the time it attains full growth, a period of about three weeks,

varying somewhat with environment. Cotyledons remaining free from infection during this period become resistant and serve as a barrier to subsequent invasion of the embryonic region of the true leaves. Consequently, if infection is prevented by environing conditions during this period of susceptibility, the plant will remain free during the remainder of its growth.

Experiments were conducted in which seedlings were grown on smut-infested soil held at various soil moisture contents. A high percentage of infected plants resulted over the entire range in which good germination and growth of the host occurred. At either extreme, very high or very low moisture, there was some reduction in amount of infection, but with it occurred a corresponding decrease in seed germination and rate of growth of the plants. Soil moisture, therefore, does not appear as a serious limiting factor in onion smut infection.

The relation of soil temperature to the development of the host and the parasite was studied by growing plants in pots held experimentally at a series of constant soil temperatures in the special apparatus known as the "Wisconsin soil temperature tank."

Seed germination and growth took place over a range of soil temperature from 10° to 31° C. Most rapid seed germination and development of tops occurred at soil temperatures of 20° to 25°, while as a rule the best development of roots occurred below 20°.

A high percentage of plants grown on smutted soil were infected at soil temperatures ranging from 10° to 25° C. A decided reduction in infection was noted at about 27°, and complete freedom from the disease resulted at 29°. In these experiments all plants were under uniform conditions of air temperature, which ranged from 15° to 20°.

The relation of variations in air temperature to the development of the disease was then studied.

Exposure of plants bearing incipient infections of the fungus in the aerial parts to an air and soil temperature of 30° to 33° C. so disturbed the relations between parasite and host as to preclude any further development of the disease. This was shown by growing plants at a temperature favorable for infection (15° to 20°). Then, just as the pustules of the disease were beginning to appear (tenth to twelfth day), the plants were removed to a room held at 30° to 33°. This stimulated top growth for a few days, which was followed by a decided checking of the plants and death after three or four weeks. However, if after 12 to 15 days at the high temperature the plants were returned to the original temperature (15° to 20°), they grew normally, but the fungus in nearly all cases failed to produce spores, and the plants remained free from further invasion.

Experiments were then performed in which seedlings were grown on infested soil held at 20°, 25°, and 30° C. with a uniform air temperature of 30° to 33°. A high percentage of infection resulted at soil temperatures

of 20° and 25°, but none at 30°, showing that high air temperature alone is insufficient to check the development of the disease. It appears probable that the failure of the fungus to complete its development in the case described above (where the plants after infection were exposed to an air and soil temperature of 30° to 33°) was brought about at least in part by some marked disturbance of the metabolism of the host and not simply by the direct effect of the high air temperature upon the fungus in the aerial parts of the seedling.

Comparison between the development of the disease in plants grown at 15° to 20° and at 24° to 28° C. (air and soil) was made. A high percentage of cotyledon infection occurred in both cases. At the lower temperature the disease proceeded as usual to the infection of the true leaves. At the higher temperature, however, the plants tended to outgrow the disease, this being associated with a more rapid rate of top development which apparently enabled the plants to slough off the smutted cotyledons before infection of the first true leaf occurred.

The foregoing conclusions as to the dominant influence of soil temperature upon onion smut infection, while primarily based on greenhouse experiments, have been found to accord well with field developments.

Successive out-of-door plantings at Madison, Wis., made in inoculated soil during the growing season, resulted in a gradual reduction of infection as the season advanced and the soil temperature rose. Complete freedom from smut was attained when the daily mean soil temperature at 1 to 2 inches depth remained at or slightly above 29° C. for two to three weeks. There was also a tendency, as the temperature rose, for the seedlings to outgrow the disease by the sloughing off of the diseased cotyledons before infection of the first leaf occurred.

An examination of records from one of the southern onion sections (Laredo, Tex.) shows that during a good share of the critical period for onion smut infection (August and September) the mean air temperature is above that at which complete inhibition of infection was attained in our experiments (29° C. or about 84° F.). If we assume, as observed in northern sections, that the mean temperature for the upper layer of soil is several degrees higher than that of the air at this time of the year, it is reasonable to conclude that even though the smut organism were introduced into southern onion sections, its development would be prevented or greatly minimized, first, by the prevention of infection due to high temperatures, and, secondly, by the rapidly developing tops outgrowing the disease, should occasional infections occur.

In general we believe, therefore, that the regional distribution of onion smut in the United States is conditioned upon the soil temperature during the seedling stage of the plant's growth, the infection and development of smut being favored by the relatively low temperatures and inhibited by the high temperatures, with approximately 29° C. as the critical point.

It is hoped that the evidence here recorded may lead to the accumulation of further field data bearing upon this particular problem by investigators in various places, especially in the southern States. It is also believed that these results illustrate well the importance of more persistent inquiry by the experimental method into the relation of environmental factors to the occurrence of disease of plants in general.

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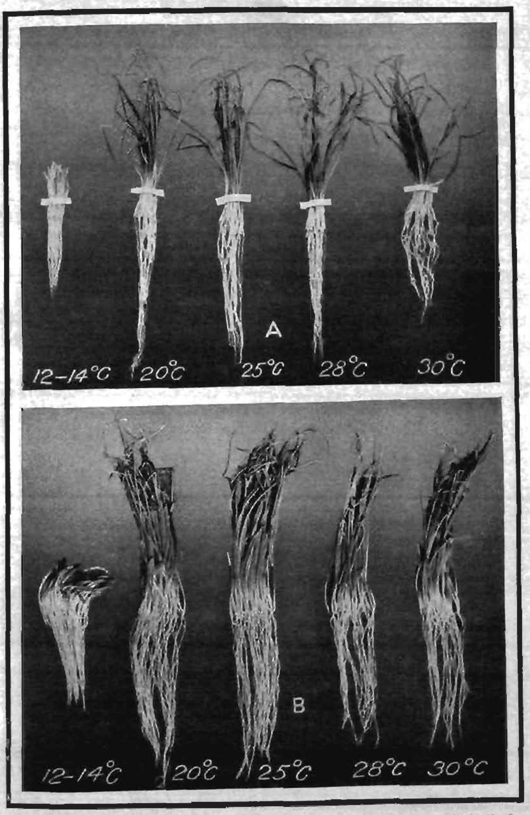
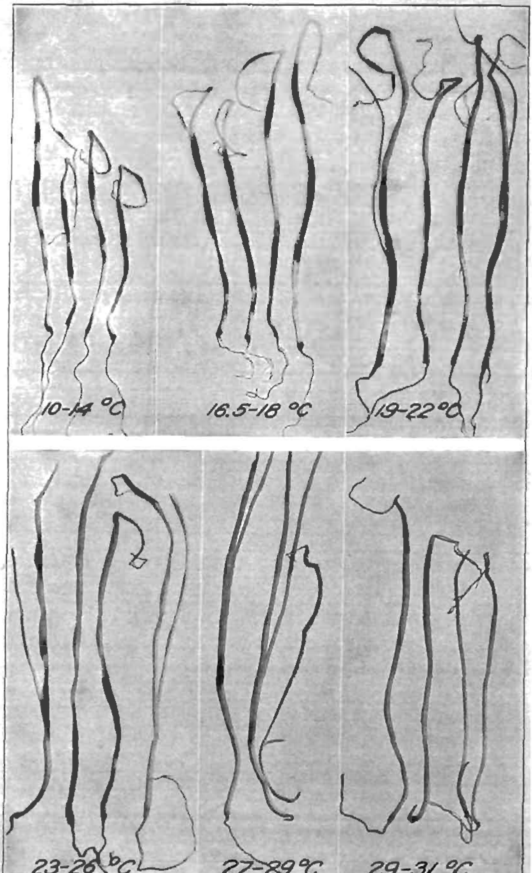
PLATE 25

Relation of soil temperature to the development of onion seedlings.

Upper row.—Seedlings of Red Globe variety 13 days old. Each cluster was the entire crop from one experimental culture pot. All were grown in like virgin soil and at the same air temperature (15° to 20° C.) but with gradation in the soil temperature of the respective pots as follows (left to right): 12° to 14°, 20°, 25°, 28°, 30°. Note that there is a tendency for greater root development in relation to top growth at the lower temperatures. This was especially marked at the lowest temperature, 12° to 14°. For further details see Table V and the accompanying text.

Lower row.—Seedlings of Yellow Bermuda variety grown under same conditions as those in upper row.


A
12–14°C
20°C
25°C
28°C
30°C
B
12–14°C
20°C
25°C
28°C
30°C


10-14 °C
16.5-18 °C
19-22 °C
23-26 °C
27-29 °C
29-31 °C

## PLATE 26

Relation of soil temperature to the infection of onion seedlings by the smut fungus, *Urocystis cepulae.*

Representative seedlings taken from the experimental culture pots, showing the influence of soil temperature upon the amount of smut. All the pots alike contained smut-infested soil. The air temperature and other aerial factors were the same for all. Soil temperature was the only factor varied experimentally, the temperature gradations extending from about 10° to 30° C.

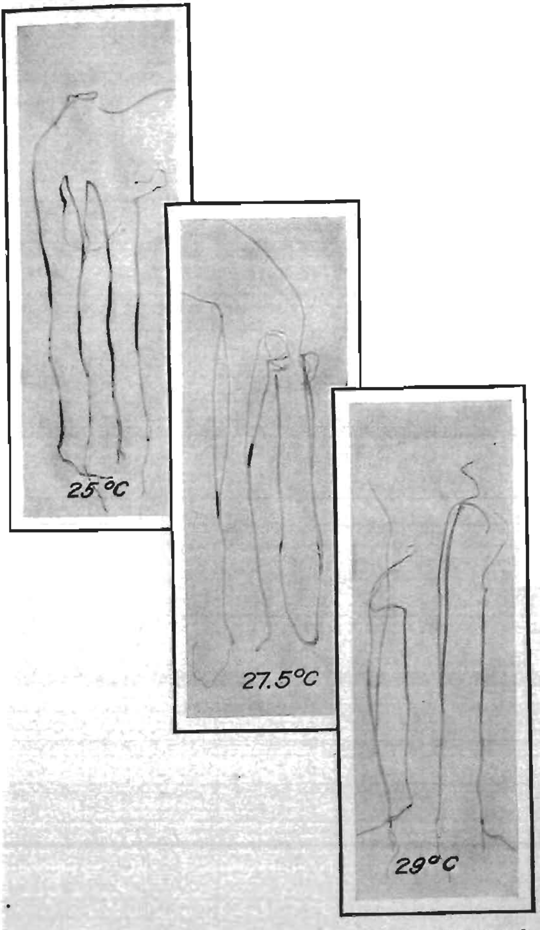
Note the abundance of smut at 10° to 22°, as shown in the upper row. A slight reduction occurred at 23° to 26°. At 27° to 29° the reduction is sharply marked. At 29° to 31° inhibition is complete. For the percentages of seedling infection and other details see Table VI and the accompanying text.

PLATE 27

Relation of soil temperature to onion smut infection.

This shows the results from a series of experiments in which the methods described for Plate 26 were repeated with the soil temperatures restricted to the critical limits between 25° and 29° C. and controlled more exactly. Note the marked reduction in infection at 27.5° and complete inhibition at 29°, thus establishing, but with more exactness, the conclusions illustrated in Plate 26.

For the percentage of infected seedlings at these temperatures and other details, see Table VIII and the accompanying text.


25°C
27.5°C
29°C

# A PHYSIOLOGICAL STUDY OF GRAPEFRUIT RIPENING AND STORAGE [1]

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In an earlier investigation (7) [2] the changes in Florida-grown grapefruit during storage were studied, particular attention being paid to the sugar and acid content of the pulp or edible portion of the fruit as influenced by some six different storage temperatures. It was found that the acid content decreased in cold storage while the total sugar content remained about the same. The percentage of cane sugar decreased and the reducing sugar content increased. At the higher temperatures, common storage (about 55° to 60°, 70°, and 86° F.) there was in some cases apparently an increase in acidity and a reduction in the amount of sugar, especially in fruit stored for long periods. The shrinkage, which was very marked in the ventilated packages at these high temperatures, made the obtaining of definite evidence on this point impossible.

The investigation described in the present paper is concerned with the acid and sugar changes in the fruit on the tree as well as with the changes which take place, both in warm storage and in cold storage, in fruit picked at monthly intervals. The control of the pitting which occurs commonly on grapefruit in cold storage is given some attention.

## PLAN OF THE EXPERIMENTS

"Common Florida" [3] fruit from two trees was picked at monthly intervals for four months, beginning July 27, making five different picks. At the last three pickings fruit was also harvested from two additional trees in the same grove. The fruit was expressed to Washington and sampled on arrival. Part of the fruit was placed in warm storage at about 70° F. and part in cold storage at 32°. Analyses were made of the fruit stored in the warm storage each month for two months and on fruit from the last three pickings stored in cold storage, after it had been in storage four months. By this plan it was possible to obtain data on the changes in the fruit on the tree from a month or so before the fruit was in condition to pick for market until the last of the season, and to compare the changes which took place in cold storage in fruit picked from the same trees at different times of the season.

[1] This paper gives the results of a portion of the work carried on under the project "Factors affecting the storage life of fruits."

[2] Reference is made by number (italic) to "Literature cited," p. 278–279.

[3] The writer's thanks are due Mr. W. J. Krome for the picking and shipping of all the "Common Florida" fruits used in these experiments.

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METHODS OF ANALYSES

The fruit was prepared for sampling and sampled as in the previous work. Analyses were made for acids, sugars, both reducing sugars and total sugars, dry weight, shrinkage, thickness of peel, and percentage of peel. In addition the acidity and specific gravity of the expressed juice of the fruits were determined and the solids-acid ratio calculated after the usual method. The acidity determinations were carried out as in the previous work, as were practically all the other determinations with the exception of the extraction of the sugar from the pulp. In the sugar extraction a method was followed similar to that described in work on potatoes (*6*). The weighed pulp was placed in a liter volumetric flask which was then filled to volume with 85 per cent alcohol. It was allowed to stand with frequent shakings for about three weeks, the losses from evaporation, of course, being made up by adding alcohol. The alcoholic solution of sugar was then separated from the residue by filtration, and the sugars were determined in aliquots of the filtrate.

The first lot of grapefruit was of small size, green in color, with very little juice in the pulp. No solids-acid determinations were made on this lot. They were, however, maintained in warm storage for two months. At the end of this period many of them had assumed the characteristic yellow color of the ripe grapefruit.

The second pick, received August 29, was much further advanced, being about 50 per cent colored and of good size. The third and fourth picks, those of October 25 and November 28, respectively, were in fine condition for shipping and are what would be considered midseason fruit. The November 28 fruit was fair, possibly a little coarser than the two picks immediately preceding. No sprouted seeds were found in any of the fruits, however. The date of picking might be considered as in the latter part of the grapefruit season for this locality and for this variety.

The analyses of grapefruit picked from trees 1 and 2, from warm storage at about 70° F. for one and two months, are shown in Tables I and II. In the analytical work the analyses were usually made in duplicate, and both analyses are given in the tables, as this furnishes evidence on the experimental error in the method of sampling. The tables are self-explanatory.

An inspection of Tables I and II shows that in the first four pickings there is in all cases an increase in the acid content of the pulp, while in the last picking from both trees there is no decided increase. A comparison of the acid content, as determined in the analyses of the pulp and the acid content of juice, shows a similar behavior. The acid content of the juice is, as a rule, markedly higher than that of the pulp, due, of course, to the fact that in the last-mentioned case the weight of fibrous material is taken into consideration in calculating the percentage

of acid. In the fifth pick from both trees there is no decided variation in the acid content of the pulp during storage, and the percentage of acid in the juice does not change as much as in fruit from any of the other four picks. With the sugars, the percentage of reducing sugars and of total sugars is always greater at the end of two months in warm storage, except in the fifth pick. The reducing sugar increases most, due probably to the inversion of some of the cane sugar which is less in all cases after two months in storage.

It was brought out in the earlier publication on grapefruit storage that there was an indication that the acid content of the fruit was slightly increased during a long period of warm storage. It was pointed out also that definite evidence on this point was difficult to obtain because the structure of the fruit prevented accurate calculation of the shrinkage of the various portions. Further evidence, mostly of an indirect nature, may be derived from the data on sugar and acid content of the fruit, found in Tables I and II. As was mentioned above, there is in all cases an apparent increase in the acid content and the total sugar content of the pulp, due for the most part undoubtedly to loss of water during storage. In the tables it is noticeable that the solids-acid ratio is usually less after two months in storage. This indicates, of course, that the increase in soluble solids is not proportional to the increase in acidity and that some soluble substance or substances other than titratable acids decreased in the storage period. This occurs in five cases out of seven on which data were obtained. The other two cases, tree 2, third pick and fifth pick, show slight increases, 0.07 and 0.11, respectively.

These data are corroborated in the total sugars-acid ratios, which are calculated by dividing the percentage of total sugar as dextrose by the percentage of acid as citric. In the 10 cases the ratio of sugar to acid is less in 7, practically the same in 2, and greater in 1. Indications are, then, that there is usually an increase in the ratio of sugar to acid under the conditions of the experiment. This could be brought about by either decreasing the sugar content of the fruit or by increasing the acid content or by a combination of these two factors. It is noticeable that in 6 cases out of 10 the acid-sugar ratio is greater after one month in storage than it is after two months at the same temperature. The acid and sugar in the fruit from warm storage will be considered later in comparison with the changes taking place in cold storage.

There is in most cases not much variation in the percentage of dry matter during storage, though there seems to be a tendency, more marked in some cases than in others, toward an increase. This seems probable, as the shrinkage where determined is from 14.4 to 23.3 per cent for the full two months in storage. The percentage of peel always decreases during storage at this temperature, due to the loss of water and wilting. This is evident in the decrease in thickness of the peel, which is very marked, especially in the earlier picks.

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CHANGES IN FRUIT ON TREES

The analyses of fruit from the various pickings at the time it was placed in storage (Tables I and II) show marked differences in composition.

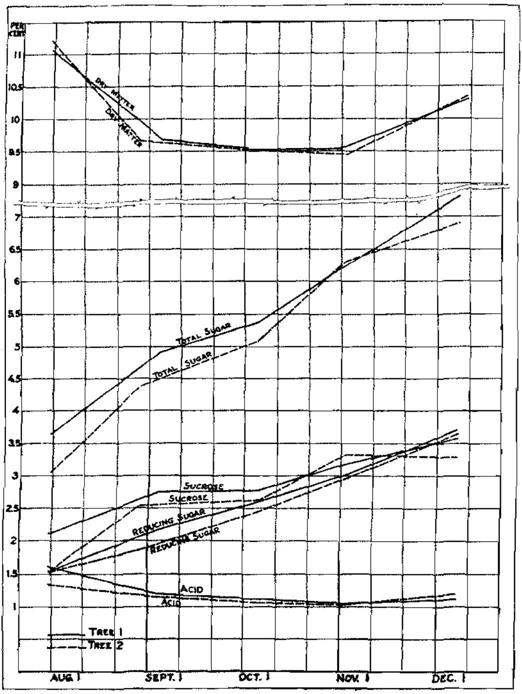


FIG. 1.—Graphs showing changes in percentage of dry matter, acid, and sugars calculated as dextrose in fruit on two Common Florida trees from August to December.

For convenience in comparison the data for sugars, acids, and dry matter are shown graphically (fig. 1). In these curves the percentage is plotted on the ordinates and the time interval between pickings on the abscissae.

TABLE I.—*Percentage of acid, sugar, and dry matter in pulp, thickness of peel and percentage of peel, acid and soluble solids in juice, and solids acid ratio of "Common Florida" grapefruit from tree No. 1, picked at monthly intervals and stored at 70° F.*

| | First pick. | | | Second pick. | | | Third pick. | | | Fourth pick. | | | Fifth pick. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Placed in storage July 20. | Second sampling Sept. 3 | Third sampling Oct. 8. | Placed in storage Sept. 5. | Second sampling Oct. 8. | Third sampling Nov. 3 | Placed in storage Oct. 4. | Second sampling Nov 4 | Third sampling Dec. 4. | Placed in storage Oct 30. | Second sampling Dec. 5. | Third sampling Jan. 3. | Placed in storage Dec. 5. | Second sampling Jan. 6. | Third sampling Feb. 6. |
| Acid as percentage of citric | 1.61<br>1.49 | 1.63<br>1.67 | 1.81<br>1.76 | 1.14<br>1.26 | 1.45<br>1.28 | 1.34<br>1.38 | 1.10<br>1.08 | 1.21<br>1.23 | 1.18<br>1.23 | 1.00<br>1.02 | 1.18<br>1.17 | 1.18<br>1.21 | 1.14 | 1.13<br>1.17 | 1.17 |
| Percentage of reducing sugar as dextrose | 1.59<br>1.46 | 1.96 | 2.99<br>3.00 | 2.17<br>2.19 | 2.91<br>2.90 | 3.27<br>3.38 | 2.62<br>2.58 | 3.05<br>2.86 | 3.48<br>3.40 | 2.96<br>3.04 | 4.71 | 4.09<br>4.07 | 3.73 | 4.43<br>4.39 | 4.48<br>4.57 |
| Percentage of cane sugar as dextrose | 2.08<br>2.18 | 2.97 | 1.20<br>1.25 | 2.62<br>2.88 | 2.57<br>2.40 | 2.08<br>2.25 | 2.61<br>2.93 | 2.86<br>2.68 | 2.48<br>2.49 | 3.13<br>3.22 | 2.31 | 2.79<br>2.50 | 3.57 | 3.56<br>3.20 | 2.90<br>2.60 |
| Percentage of total sugar as dextrose | 3.67<br>3.64 | 4.93 | 4.10<br>4.25 | 4.79<br>5.07 | 5.48<br>5.30 | 5.35<br>5.63 | 5.23<br>5.51 | 5.91<br>5.54 | 5.96<br>5.89 | 6.09<br>6.26 | 7.02 | 6.88<br>6.57 | 7.30 | 7.99<br>7.59 | 7.38<br>7.17 |
| Total sugar-acid ratio | 2.35 | 2.98 | 2.35 | 4.10 | 3.96 | 4.03 | 4.92 | 4.68 | 4.93 | 6.1 | 6.0 | 5.63 | 6.4 | 6.77 | 6.21 |
| Percentage of dry matter | 11.20<br>11.38 | 11.44<br>11.30 | 11.27 | 9.75<br>9.59 | 9.72 | 9.50<br>9.63 | 9.49<br>9.63 | 9.28<br>9.13 | 9.66<br>9.62 | 9.45 | 10.24 | 10.10 | 10.32<br>10.35 | ... | 10.32<br>10.81 |
| Percentage of peel | 33.3 | 32.3 | 26.1 | 25.6 | 23.2 | 22.7 | 23.3 | 19.9 | 19.8 | 21.8 | ...... | 16.7 | 21.2 | 14.3 | 15.8 |
| Thickness of peel | 6.8 | 5.2 | 5.0 | 6.1 | 4.8 | 4.9 | 5.5 | 3.8 | 3.9 | 5.0 | 3.0 | 2.6 | 4.5 | 2.2 | 2. |
| Percentage of shrinkage | ... | ... | ... | ...... | ... | ... | ... | ... | ... | ... | 14.4 | 14.4 | ...... | ... | 23 |
| Acidity of juice as percentage of citric acid | ... | ... | ... | 1.44 | 1.56 | 1.65 | 1.38 | 1.11 | 1.35 | 1.14 | 1.45 | 1.46 | ... | 1.4 | 1.3 |
| Soluble solids (Brix) | ... | ... | ... | 9.21 | 10.29 | 9.99 | 9.45 | 10.61 | 9.81 | 10.11 | 11.52 | 11.47 | ... | 11.81 | 11.47 |
| Solids-acid ratio | ... | ... | ... | 6.36 | 6.61 | 6.05 | 7.38 | 9.50 | 7.23 | 8.81 | 7.93 | 7.87 | ... | 8.45 | 8.80 |

TABLE II.—*Percentage of acid, sugar, and dry matter in pulp, thickness of peel and percentage of peel, acid and soluble solids in juice, and solids-acid ratio of "Common Florida" grapefruit from tree No. 2, picked at monthly intervals and stored at 70° F.*

| | First pick. | | | Second pick. | | | Third pick. | | | Fourth pick. | | | Fifth pick. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Placed in storage July 29. | Second sampling Sept. 4. | Third sampling Oct. [illegible] | Placed in storage Sept. 3. | Second sampling Oct. [illegible] | Third sampling Nov. 3. | Placed in storage Oct. [illegible] | Second sampling Nov. [illegible] | Third sampling Dec. [illegible] | Placed in storage Nov. 1. | Second sampling Dec. 8. | Third sampling Jan. 5. | Placed in storage Dec. [illegible] | Second sampling Jan. 5. | Third sampling Feb. [illegible] |
| Acid as percentage of citric | 1.35 | 1.51<br>1.57 | 1.13<br>1.65 | 1.10<br>1.2 | 1.2[illegible]<br>1.29 | 1.56<br>1.5 | 1.00 | 1.14 | 1.2[illegible]<br>1.26 | 1.06<br>.98 | 1.10<br>1.17 | 1.23 | 1.15 | 1.12<br>1.13 | 1.21 |
| Percentage of reducing sugar as dextrose | 1.48<br>1.56 | 2.51<br>2.16 | 2.79 | 1.78<br>1.9[illegible] | [illegible].93 | 3.45<br>3.18 | 2.17<br>2.14 | 2.68 | 3.48<br>3.40 | 3.01<br>[illegible].92 | 3.59<br>3.51 | 4.26<br>4.46 | 3.73<br>[illegible].66 | 4.09 | 4.39<br>4.35 |
| Percentage of cane sugar as dextrose | 1.52<br>1.54 | 2.04<br>2.28 | 1.43 | [illegible].52<br>2.54 | 2.11 | 2.06<br>2.36 | 2.74<br>2.52 | 2.20 | 2.16<br>2.03 | [illegible].30<br>3.38 | 2.73<br>2.89 | 2.79<br>2.68 | 3.24<br>3.25 | 4.65 | 2.86<br>2.78 |
| Percentage of total sugar as dextrose | 3.00<br>3.10 | 4.55<br>4.44 | 4.22 | 4.30<br>4.46 | 5.04 | 5.51<br>5.54 | 5.21<br>4.96 | 4.97 | 5.64<br>5.43 | 6.31<br>6.30 | 6.32<br>6.40 | 7.05<br>7.14 | 6.97<br>6.91 | 8.74 | 7.25<br>7.13 |
| Total sugar-acid ratio | 2.25 | 2.91 | 2.57 | 3.71 | 4.0 | 3.6 | 4.79 | 4.35 | 4.42 | 6.17 | 5.48 | 5.76 | 6.03 | 7.80 | 5.94 |
| Percentage of dry matter | 11.13<br>11.05 | 11.24<br>11.46 | 11.22 | 9.72<br>9.59 | 9.60 | 10.53<br>10.46 | 9.54 | 9.80 | 9.55 | 9.66<br>9.43 | 10.36<br>10.23 | 11.16<br>11.15 | 10.30 | 10.09 | 10.18 |
| Percentage of peel | 45.8 | 34.8 | 28 | 29.1 | 24.2 | 24.1 | 23.5 | 25.8 | 20.4 | 21.2 | 21.8 | 13.2 | 18.2 | 13.7 | 14.7 |
| Thickness of peel | 8.1 | 7.7 | 5.1 | 6.9 | 4.6 | 5.0 | 5.6 | 5.3 | 4.3 | 4.2 | 3.8 | 2.9 | 3.[illegible] | 3.0 | 2.3 |
| Percentage of shrinkage | .. | .... | .... | .... | .... | ... | .... | .... | ... | . | 19.6 | 19.5 | . | 10.1 | 18.0 |
| Acidity of juice as percentage of citric | ... | | ..... | 1.44 | 1.59 | 1.68 | 1.29 | 1.41 | 1.5 | 1.18 | 1.37 | 1.51 | 1.10 | 1.28 | 1.28 |
| Soluble solids in juice (Brix) | .. | | .. | 9.27 | 10.6 | 9.92 | 8.15 | 9.55 | 9.59 | 10.33 | 10.59 | 10.97 | 10.79 | 10.77 | 11.67 |
| Solids-acid ratio | | | .. | 6.41 | 6.63 | 5.88 | 6.31 | 6.74 | 6.39 | 8.73 | 7.97 | 7.26 | 9.03 | 8.41 | 9.14 |

From an inspection of the curves it is evident that there is a decrease in acidity as the season advances, the acid being highest in both cases at the beginning of the season. The acid content is lowest at the fourth pick and rises slightly at the fifth pick. Collinson (3) shows a somewhat similar decrease in acidity. This writer analyzed the fruit at more frequent intervals but apparently did not begin sampling so early in the season. According to his work there is a general tendency toward lower acidity, though in a few of his series of analyses there is a higher acidity toward the end of the season than in the midseason fruit.

There is a rise in the percentage of total sugars during the season, the total sugar content of pulp of the fifth pick being about double that of the first pick. Collinson shows an increase in the total sugar content, but it is not so marked, due probably to the fact that his series begins later in the season. As shown in the curves (fig. 1), the rise in total sugar content during the first month is very sharp. The increase in percentage of reducing sugar during the season is much more gradual and regular than that of the total sugars. The percentage of this sugar in the pulp a little more than doubles in the four months of the experiment. Much the same ratio of increase is found in the total sugars. The cane sugar curves are not so regular. There is, however, a marked increase in the percentage of cane sugar. The mean of the two sucrose curves is always higher than that of the reducing sugars except at the last sampling. Collinson records a series of analyses in which the reducing sugar is markedly higher than the sucrose during the latter part of the season. The irregularities in the total sugar curves in the present work are due to the variation in sucrose content.

The percentage of dry matter, as determined in this work, is highest at the first of the season between 11 and 11.5 per cent but drops in the first month to between 9.5 and 10 per cent, the third and fourth analyses giving about the same results. There seems to be, however, an increase in the dry weights in the last month.

A comparison of the percentage of peel (Tables I and II) at the time the five different lots of fruit were placed in storage shows there is a decrease from 33.3 to 21.2 and 45.6 to 18.2 per cent of peel by weight for trees No. 1 and 2, respectively. The percentage of peel decreases much more rapidly in the first month than in the succeeding month. In fact, in the case of tree 1 there is only a slight decrease in the proportion of peel to pulp in the last three pickings. At the time these last three pickings were made, the fruit was ready for market.

As might be expected, the decrease in thickness of the peel, as measured in these experiments, parallels the decrease in percentage of peel. The peel was found to be 6.8 mm. and 8.1 mm. thick, respectively, for trees 1 and 2 when the first pick was placed in storage, while it measured 4.5 mm. and 3.2 mm. at the first sampling of the fifth pick. This is a reduction of 34 per cent and 60 per cent in the thickness of the skin for

the season. It is evident that the proportion of peel to pulp and thickness of the peel decrease as the fruit matures.

A comparison of the acid and sugar changes in grapefruit in growth and ripening with the acid and sugar changes of other fruits in the same period of their life history brings out some interesting correlations and differences. The total sugar content of deciduous fruits usually increases during the growing and ripening period. This has been shown for apples by Lindet, (*8*) Bigelow, Gore, and Howard (*2*), and others; for pears by Ritter (*12*), Riviere and Bailhache (*11*), Magness (*10*), and by Bigelow and Gore (*1*), for peaches. Numerous other investigations corroborating this point might be mentioned. The literature pertinent to this subject may be found in the works referred to here. With certain vegetables a somewhat similar increase in total sugars is found. This was brought out by Hasselbring (*5*), working with sweet potatoes, and Sando (*14*) with tomatoes. This evidence would seem to indicate that in fruits or vegetables where sugar is stored the percentage of sugar calculated on a wet-weight basis increases during the growing season—that is, there is not only an absolute increase but an increase in proportion of sugar present as compared to the sum of the other constituents. This increase in some cases is due to an increased content of reducing sugars, as in the tomato, or may be due to an increase in both reducing sugars and cane sugar, as in the apple, pear, and peach, or for the most part to an increase in cane sugar, as in the sweet potato.

In regard to the acid content of fruits which contain both sugar and acids in appreciable quantities, there is sometimes an increase and sometimes a decrease in acidity as the season advances. In pears there is generally a decrease. Magness (*10*), however, found that pears from the Yakima district, Washington, and Medford district, Oregon, showed an increased acidity as the season advanced. Apples, according to the analyses of Lindet (*8*), and Bigelow, Gore, and Howard (*2*), exhibit a decrease in acidity as the growing season advances. Peaches, on the other hand, increase in acid content as they approach maturity. The decrease in acidity of grapefruit during the growing season is comparable to the usual behavior of the acidity in pears and in apples.

## COLD STORAGE EXPERIMENTS

As mentioned earlier in this article, experiments on the cold storing of grapefruit were carried out during the 1920–21 season. Fruit from four trees was used. These trees included the two from which fruit was obtained for the warm storage work, the fruit being from the lots designated third, fourth, and fifth picks in the experiments already described. Not sufficient fruit was available for this work from the fifth pick from tree 1, so only two experiments were possible with fruit from this tree.

Table III gives the results of analyses made at the time the fruit was placed in storage and four months later. As was pointed out, because of the structure of the fruit it is very difficult to obtain definite evidence on the changes of the various constituents of the pulp. While the

fruit for each experiment was carefully selected from a lot of fruit all harvested at the same time from a single tree, the variation in composition of the fruit on this tree introduces a possible error which it is hardly possible to calculate. It is only by obtaining a large amount of evidence that a clear indication of the direction of the change can be obtained. It was accordingly deemed advisable to give in this table all the data obtained in the analyses in the 11 different storage experiments carried out in this portion of the investigation. The table is self-explanatory.

TABLE III.—*Percentage of acid, sugar, and dry matter in pulp, thickness of peel and percentage of peel, acid and soluble solids in juice, and solids-acid ratio of "Common Florida" grapefruit before and after storing four months at 32° F.*

TREE 1

| | First lot. | | Second lot. | | Third lot. | |
|---|---|---|---|---|---|---|
| | When placed in storage. | After 4 months in storage. | When placed in storage. | After 4 months in storage. | When placed in storage. | After 4 months in storage. |
| Acid as percentage of citric | 1.10<br>1.08 | 0.88<br>.90 | 1.00<br>1.02 | 0.88<br>.92 | . . .<br>. . . | . . .<br>. . . |
| Percentage of reducing sugar as dextrose | 2.62<br>2.58 | 2.55<br>2.74 | 2.96<br>3.04 | 3.48<br>3.09 | . . .<br>. . . | . . .<br>. . . |
| Percentage of cane sugar as dextrose | 2.61<br>2.93 | 3.11<br>2.72 | 3.13<br>3.22 | 3.11<br>3.51 | . . .<br>. . . | . . .<br>. . . |
| Percentage of total sugar as dextrose | 5.23<br>5.51 | 5.66<br>5.46 | 6.09<br>6.26 | 6.59<br>6.6 | . . .<br>. . . | . . .<br>. . . |
| Percentage of dry matter | 9.49<br>9.63 | 9.43<br>9.37 | 9.45<br>9.54 | 9.74<br>9.74 | . . .<br>. . . | . . .<br>. . . |
| Percentage of peel | 23.3 | 26.9 | 21.8 | 21.6 | . . . | . . . |
| Thickness of peel (in mm.) | 5.5 | 5.5 | 5 | 4.3 | . . . | . . . |
| Percentage of shrinkage | . . . . | 5 | . . . . | 4.9 | . . . | . . . |
| Acidity of juice as percentage of citric | 1.27 | 1.02 | 1.14 | 1.01 | . . . | . . . |
| Soluble solids (Brix) | 9.45 | 8.87 | 10.11 | 11.25 | . . . | . . . |
| Solids-acid ratio | 7.38 | 8.66 | 8.81 | 11.16 | . . . | . . . |

TREE 2

| | | | | | | |
|---|---|---|---|---|---|---|
| Acid as percentage of citric | 1.06 | 0.98 | 1.06 | 1.00 | 1.15 | 1.10 |
| Percentage of reducing sugar as dextrose | 2.47<br>2.44 | 2.61 | 3.01<br>2.92 | 3.37<br>3.54 | 3.73<br>3.66 | 3.29 |
| Percentage of cane sugar as dextrose | 2.74<br>2.52 | 2.65 | 3.30<br>3.38 | 3.03<br>2.75 | 3.24<br>3.25 | 3.59 |
| Percentage of total sugar as dextrose | 5.21<br>4.96 | 5.26 | 6.31<br>6.30 | 6.40<br>6.29 | 6.97<br>6.91 | 6.88 |
| Percentage of dry matter | 9.54 | 9.46 | 9.66<br>9.43 | 9.86<br>10.16 | 10.30 | 9.77<br>9.82 |
| Percentage of peel | 23.5 | 23.3 | 21.2 | 21.7 | 18.2 | 22.4 |
| Thickness of peel (in mm.) | 5.6 | 4.4 | 4.2 | 4.3 | 3.2 | 4.8 |
| Percentage of shrinkage | . . . . . . . . | 3 | . . . . . . . . | 4 | . . . . . . . . | 3.9 |
| Acidity of juice as percentage of citric | 1.28 | 1.19 | 1.18 | 1.09 | 1.19 | 1.05 |
| Soluble solids (Brix) | 9.40 | 10.04 | 10.33 | 10.16 | 10.79 | 11.35 |
| Solids-acid ratio | 7.31 | 8.44 | 8.73 | 10.29 | 9.03 | 10.84 |

TABLE III.—*Percentage of acid, sugar, and dry matter in pulp, thickness of peel and percentage of peel, acid and soluble solids in juice, and solids-acid ratio of "Common Florida" grapefruit before and after storing four months at 32° F.*—Continued

TREE 3

| | First lot. | | Second lot. | | Third lot. | |
|---|---|---|---|---|---|---|
| | When placed in storage. | After 4 months in storage. | When placed in storage. | After 4 months in storage. | When placed in storage. | After 4 months in storage |
| Acid as percentage of citric | 1.06 | 0.98 | 1.10<br>1.09 | 1.03<br>1.07 | 1.29<br>1.18 | 1.12<br>1.13 |
| Percentage of reducing sugar as dextrose | 2.46<br>2.58 | 2.55<br>2.35 | 2.77<br>2.50 | 3.22<br>3.50 | 3.36<br>3.51 | 3.31<br>3.51 |
| Percentage of cane sugar as dextrose | 2.83<br>3.01 | 2.58<br>2.77 | 2.81<br>2.81 | 3.16<br>2.75 | 3.01<br>3.25 | 2.86<br>2.88 |
| Percentage of total sugar as dextrose | 5.29<br>5.59 | 5.13<br>5.12 | 5.58<br>5.31 | 6.38<br>6.25 | 6.37<br>6.76 | 6.17<br>6.39 |
| Percentage of dry matter. | 8.77<br>8.78 | 8.10<br>8.53 | 9.56<br>9.16 | 9.19<br>9.67 | 9.82<br>9.68 | 10.12<br>10.26 |
| Percentage of peel | 24 | 24.2 | 22.2 | 21.8 | 22.2 | 20.3 |
| Thickness of peel (in mm) | 5.3 | 4.7 | 4.9 | 4 | 4.3 | 4 |
| Percentage of shrinkage | ........ | 5 | ........ | 4.3 | ........ | 4.6 |
| Acidity of juice as percentage of citric. | 1.26 | 1.17 | 1.25 | 1.08 | 1.29 | 1.17 |
| Soluble solids (Brix). | 8.65 | 11.19 | 9.66 | 9.46 | 10.29 | 11.19 |
| Solids-acid ratio | 6.92 | 9.51 | 7.71 | 8.73 | 7.94 | 9.51 |

TREE 4

| | First lot: When placed in storage. | First lot: After 4 months in storage. | Second lot: When placed in storage. | Second lot: After 4 months in storage. | Third lot: When placed in storage. | Third lot: After 4 months in storage |
|---|---|---|---|---|---|---|
| Acid as percentage of citric | 1.18<br>1.10 | 0.94<br>1.03 | 1.24 | 1.04 | 1.21<br>1.22 | 1.06<br>1.18 |
| Percentage of reducing sugar as dextrose | 2.70<br>2.66 | 2.18<br>2.80 | 3.04 | 3.21<br>3.23 | 3.75<br>3.85 | 3.28<br>3.30 |
| Percentage of cane sugar as dextrose | 2.62<br>2.56 | 3.31<br>2.60 | 1.78 | 3.03<br>3.16 | 3.10<br>3.02 | 2.78<br>2.49 |
| Percentage of total sugar as dextrose | 5.32<br>5.22 | 5.49<br>5.49 | 5.82 | 6.24<br>6.39 | 6.85<br>6.87 | 6.06<br>5.79 |
| Percentage of dry matter | 9.28<br>9.53 | 9.91 | 9.58<br>9.57 | 9.97<br>9.96 | 9.87<br>9.90 | 9.79<br>9.94 |
| Percentage of peel | 22 | 26.4 | 22.3 | 21.7 | 21 | 24.5 |
| Thickness of peel (in mm) | 5.4 | 5.3 | 4.5 | 4.9 | 4 | 5.3 |
| Percentage of shrinkage | ........ | 4.9 | ........ | 3.3 | ........ | 4 |
| Acidity of juice as percentage of citric | 1.36 | 1.17 | 1.31 | 1.04 | 1.26 | 1.18 |
| Soluble solids (Brix) | 9.35 | 10.01 | 10.23 | 10.16 | 10.86 | 10.59 |
| Solids-acid ratio | 6.86 | 8.55 | 7.79 | 9.73 | 8.60 | 8.90 |

From Table III it is evident that in every case there is a lower acidity in the fruit after it has been held in storage four months than in fruits from the same tree and picking when placed in storage. This is in accordance with the findings reported in the previous publication and would seem to establish this point definitely. The fact that in the present experiments fruit was picked at three different times during the growing and ripening season strengthens the evidence.

The total sugar content is usually slightly higher at the end of the four months' storage period, though there are several instances in which it is lower. These cases are mostly in well-matured fruit of the last pick. The increase in total sugars is due for the most part to an increase in the reducing-sugar content, as there is usually a marked decrease in the percentage of cane sugar during storage. There is never more than 5 per cent shrinkage during these four months. This shrinkage is doubtless partly from the peel and partly from the interior portion or pulp.

The fact that in most cases there is an apparent increase in total sugars can be accounted for by the loss of water and consequent shrinkage. It is very evident from these data that there is no appreciable diminution in the amount of sugar in the grapefruits in four months at 32° F. On the other hand, there is without doubt no considerable increase. It is, of course, probable that some of the pectins and other hemicelluloses or the glucosid in the fruit break down slowly, and it is possible that some reducing substance is formed from these decomposition products.

A comparison of the behavior of the acids and sugars in grapefruits stored in warm storage (Tables I and II) with the results obtained in the cold storage experiments just considered brings out some rather striking differences. In the data obtained from the warm storage experiments there is evidence of an increase in acidity or a decrease in total sugars or both—that is, in most cases the ratio of total sugar to acid decreases, while in the cold storage the reverse is true. This is corroborated by the acidity and soluble solids of the juice. In the warm storage experiments the solids-acid ratio is in most cases less after two months in storage, while in the cold storage there is always a decrease in acidity and an increase in solids-acid ratio. It is evident that there is an increase, or at least not a decrease, in acidity in warm storage and a decided decrease in cold storage. It would, therefore, seem probable that some of the processes which go on in the fruit stored in the warm are modified when the fruit is placed in cold storage. It is possible, of course, that in respiration the acid is used up in cold storage while the sugars are used in warm storage. There is an indication that the sugar content may decrease slightly in the fruits held in warm storage, while there is no evidence of change in the percentage of sugar in the cold-stored fruits. Magness (*9*) has shown that the composition of the gases in the interior of apples and potatoes varies with the temperature at which they are held. For example, he found that the gas from the interior of Yellow Newtown apples stored at 2° C. (about 35° F.) analyzed 14.2 per cent $O_2$ and 6.7 per cent $CO_2$, while at 30° C. (86° F.) the extracted gas was 3.2 per cent $O_2$ and 21.4 per cent $CO_2$. The air surrounding the fruits used in these experiments was practically the same in both cases. The oxygen content was low and the carbon-dioxid content high in the fruit at high temperatures because the oxygen was used up in respiration faster than it could diffuse in from the outside. While no such determinations have been made on grapefruits, it seems

probable from the size of the fruit and the thickness and structure of the peel that in fruit held at high temperatures for any considerable period there would be a low oxygen pressure. This might result in some intermolecular respiration and the formation of acid. At low temperatures the respiration rate would be considerably decreased, while the rate of diffusion of $O_2$ through the tissues would not be so greatly reduced, and sufficient oxygen might be present for the breaking down of the compounds used in respiration of $CO_2$ and $H_2O$. A careful investigation of this point is needed. The work of Gerber (*4*) is of interest in this connection.

The dry weights are about the same at the conclusion of the experiments as at the beginning. There may be a slight diminution in the percentage of dry matter, but this apparently lies within the experimental error of the determinations. The variation in thickness of pee and percentage of peel is so great that there is frequently a higher percentage of peel after the fruit has been stored four months than when i was placed in storage. This is undoubtedly due to the lack of uniformity in the fruits and the low percentage of shrinkage.

The loss in weight during the four months' storage is from 3 to 4.9 pe cent, averaging around 4 per cent. The relative humidity of the storag rooms was around 75 per cent. The fruit was not in the best conditio for merchandising at the end of this storage period, as it was in man cases badly pitted. It is doubtful whether this method of storage woul be applicable to commercial conditions if the fruit were placed directl in cold storage.

## EXPERIMENTS IN THE CONTROL OF PITTING

As was mentioned in the earlier paper (*7*), grapefruit tends to pit i cold storage. This pitting begins as a small indentation of the skin i practically any region of the surface. The sunken area gradually increases in size, frequently becoming as much as 1 cm. in diameter. The are usually, in the type of fruit used in these experiments, about 1 mm in depth. In time they may take on a brown color. This coloring occur more quickly if the fruit is removed to a warm room. These pits may b very numerous on the surface of the fruit, in many cases coalescing i irregular shaped patches.

Cross sections of these pits show that they are formed by a breakin down of the layer of tissue containing the oil vesicles. There is apparently no disintegration of the tissue. The cells and vesicles simply flatte out as if subjected to local pressure, the layer of tissue becoming brown The injury apparently does not extend to any distance in the spong tissue beneath this oil-bearing layer, and it is only after a long period tha any evidence of the discoloration appears on the inside of the peel. Th pulp of the fruit is apparently uninjured. The affected fruit, however is very unsightly, and badly pitted fruit would hardly be salable in a normal market. It was evident that unless some method of preventin

this pitting was worked out the storing of grapefruit for any considerable period would hardly be commercially practicable.

Experiments were, therefore, undertaken to see if it were possible to treat or handle the fruit so that it could be cold-stored without this danger of pitting. As was mentioned earlier, fruit stored in warm storage, 70° to 86° F., or in common storage (about 55° to 60°) apparently does not pit. It was considered possible that if fruit were cured for a time in warm storage before being placed in cold storage the injury from this blotching and breaking down of the surface of the peel might be obviated. Accordingly a lot of 1 dozen fruits from tree 1, third pick, was maintained at a temperature of 70° and a humidity of about 65 per cent for one month, then removed to cold storage (32°) and xamined at intervals. At the end of three months in cold storage none of these fruits were pitted, while about 60 per cent of the fruit from the same lot placed directly in cold storage at 32° were badly pitted.

The experiment was repeated with grapefruits of the Duncan, Marsh Seedless, and Silver Cluster varieties from Polk County, Fla., which were placed in storage February 12, 1920. Part of the fruit of each lot was placed directly in 32° F., and the rest of the three lots were placed in the curing room and maintained at a temperature of about 70° with a relative humidity around 60 per cent. Portions of the lots from the curing room were removed to 32° cold storage at intervals. The entire storage period was three months for all lots. The results of the experiment are shown in Table IV, in which are given the length of time in curing, the time in cold storage, and the percentage of pitting of the different lots. In these experiments the pitting is given as slight and bad pitting. Bad pitting is applied to pitting that would markedly injure the sale of the fruit. Slight pitting refers to pitting that while noticeable does not particularly injure the fruit for sale. It is at most a few spots usually small.

It is noticeable in Table IV that most of the control fruit that was placed directly in cold storage without curing is pitted and that there is a high percentage of bad pitting. In the Duncan, 6 per cent was good, while the poorest lot of cured fruit of this variety was about 90 per cent good. There was more pitting in the cured Silver Cluster than in the Duncan and somewhat more in the Marsh Seedless than in the Silver Cluster. The data obtained in this one storage experiment are hardly sufficient, however, to justify the conclusion that Duncan grapefruit store better than Silver Cluster and Marsh Seedless. The experiments, however, seem to show that the pitting can be controlled by proper curing before the fruit is placed at the low temperatures. The specific effect of this curing, by exposure to warm temperatures from one to six weeks, on the tissue of the peel so that the pitting is prevented has, of course, received little attention. Pitting has all the external appearance of injury considered to be due to *Colletotrichum gloeosporioides* (Penz.) by Rolfs, Fawcett, and Floyd (*13*) and figured by them. This fungus,

however, has a high optimum temperature, and it seemed highly improbable that its growth could be controlled by exposing to high temperatures and that it caused most damage at temperatures around 32° to 40° F. It was possible, however. This point was investigated by Dr. F. V. Rand, of the Laboratory of Plant Pathology, Bureau of Plant Industry. The results of this work are as yet unpublished. The following account, however, is based on Dr. Rand's work.

TABLE IV.—*Results of storage experiments with Duncan, Marsh Seedless, and Silver Cluster grapefruit*

| Number of fruits. | Number of days in curing room. | Number of days in cold storage. | Temperature of cold storage. | Number of good fruits. | Percentage of good fruit. | Number with slight pitting. | Percentage with slight pitting. | Number with bad pitting. | Percentage with bad pitting. |
|---|---|---|---|---|---|---|---|---|---|
| DUNCAN | | | | | | | | | |
| | | | °F. | | | | | | |
| 50 | [a] 00 | 90 | 32 | 3 | 6 | 10 | 20 | 37 | 74 |
| 27 | 12 | 78 | 32 | 24 | 88.9 | 3 | 11.1 | ... | ... |
| 23 | 19 | 71 | 32 | 23 | 100 | ... | ... | ... | ... |
| 14 | 25 | 65 | 32 | 13 | 92.8 | 1 | 7.1 | ... | ... |
| 10 | 41 | 49 | 32 | 10 | 100 | ... | ... | ... | ... |
| MARSH SEEDLESS | | | | | | | | | |
| 82 | [a] 00 | 90 | 32 | 3 | 3.6 | 6 | 7.3 | 73 | 89 |
| 42 | 10 | 80 | 32 | 11 | 26.2 | ... | ... | 31 | 73.8 |
| 30 | 18 | 72 | 32 | 12 | 40 | 6 | 20 | 12 | 40 |
| 19 | 24 | 66 | 32 | 16 | 84.2 | 3 | 15.8 | ... | ... |
| 12 | 30 | 60 | 32 | 10 | 83.3 | 2 | 16.7 | ... | ... |
| SILVER CLUSTER | | | | | | | | | |
| 46 | [a] 00 | 90 | 32 | 23 | 50 | ... | ... | 23 | 50 |
| 16 | 9 | 81 | 32 | 14 | 87.5 | 2 | 12.5 | ... | ... |
| 17 | 18 | 72 | 32 | 15 | 88.2 | 2 | 11.8 | ... | ... |
| 20 | 25 | 65 | 32 | 17 | 85 | 3 | 15 | ... | ... |

[a] Controls placed directly in cold storage.

Cultures were made from the pits and from the tissue of the peel between the pits. Cultures were also made from the peel of cured fruits which had been in cold storage for three months after curing. The results are shown in Table V.

It is evident from Table V that Colletotrichum was almost universally present in the peel of these Florida grapefruits and that while it is usually to be found in the pit it is just as common in the normal peel of the pitted fruit or the cured fruit. It is, of course, impossible to assert from the evidence at hand that the fungus does not cause the breaking down of the peel. The cold storage might so affect the physiology of the peel as to make it susceptible to fungus attacks, while curing

and warm storage render it resistant. This, of course, is somewhat doubtful. The case is somewhat analagous to that cited by Winston (*15*) in regard to tear stain, which has up to now been considered to be due to *Colletotrichum gloeosporioides*, mainly because this fungus was usually found in cultures from the diseased areas. In the present work it seems fair to conclude that whether or not the fungus causes the pitting it is controlled at least to a large extent by curing before placing the fruit in cold storage.

TABLE V.—*Results of cultural experiments with pitted grapefruit and with fruit from same lot which was unpitted* [a]

| Date. | Source. | Number of fruits. | Number of pieces of tissue | Colletotrichum. | Cladosporium. | Alternaria. | Fusarium. | Penicillium. | Sterile. | Miscellaneous fungi. |
|---|---|---|---|---|---|---|---|---|---|---|
| July 7, 1920 | Pits...... | 6 | 96 | 33 | 31 | 1 | ...... | ...... | 31 | ...... |
| | Between pits .. | 1 | 7 | 1 | 5 | | ...... | ...... | 1 | ...... |
| Aug. 9, 1920 | Pits .. | 2 | 33 | 17 | ...... | ...... | ...... | ...... | 16 | ...... |
| | Between pits .... | 2 | 17 | 10 | . | | .... | ..... | 7 | ...... |
| Feb. 1, 1921 | Pits ... | 7 | 53 | 30 | 3 | .. | .... | 1 | 21 | 1 |
| | Between pits .. | 4 | 29 | 15 | 3 | .... | . | 3 | 8 | 6 |
| | Control.. | 5 | 70 | 42 | .. | . | .... | 4 | 22 | 4 |
| Feb. 1, 1921 (not sterilized) | Pits ... | 3 | 32 | 21 | ...... | .. | .. | 21 | ... | 13 |
| | Between pits .... | 2 | 27 | 12 | | . | . | 11 | | 27 |
| | Control . | 1 | 14 | 14 | | | . | 10 | ...... | 13 |
| Feb. 3, 1921 (sterilized 3 minutes). | Pits .... | 3 | 38 | .. | | .. | . | ..... | 3[b] .. | .. |
| | Between pits ... | 3 | 17 | 3 | | . | 1 | . | 13 | |
| | Control. . | 3 | 23 | 16 | . . | | 1 | .. | 6 | .. |
| Feb. 3, 1921 (not sterilized) | Pits. . . | 4 | 38 | 38 | | . | 2 | . | .. .. | 33 |
| | Between pits ... | 2 | 24 | 15 | . . | | . | 2 | | 7 |
| | Control | 2 | 20 | 17 | . . | . | | 1 | . | 3 |
| Feb. 8, 1921. | Pits ... | 5 | 93 | 68 | . .. | 6 | 7 | . | 10 | 10 |
| | Between pits. .. | 2 | 18 | 27 | | 3 | ... | 1 | 4 | 2 |
| | Control... | 4 | 84 | 63 | ..... | 5 | 5 | .... | 10 | 3 |

[a] Unless otherwise stated, all pieces of tissue were sterilized two minutes in 1 to 1,000 bichlorid solution and were washed three times in sterile tap water before pouring plates. The control fruits were without signs of the pitted spots under investigation. "Between pits" refers to sound tissue between the spots.

## GENERAL DISCUSSION AND CONCLUSION

In the investigation of grapefruit storage described in the foregoing pages it has been brought out that in warm storage the percentage of acid calculated to the wet weight of the pulp increases markedly in two months' storage. There is evidence that this increase is not due entirely to loss of water from the pulp, but that there is an increase in the amount of acid present. There is evidence indicating that there may be a slight decrease in the sugar content in warm storage. In cold storage there is a decrease in the acidity very marked after four months in storage, while there is little change in the amount of total sugars present. A possible explanation of this difference in the behavior of the sugars and acids in warm and cold storage was pointed out. This phase of the problem deserves further attention. The investigations on the changes in the

fruit during development on the tree showed that the total sugar content increased while the acidity decreased, the increase in sugar content being very marked.

Fruit on the tree increases in palatability and food value. There is, of course, always danger that the seeds will sprout in the varieties containing seeds if the fruit remains on the tree too long. There is also danger that the fruit will drop or be shaken from the tree by high winds.

It is of interest to note that the behavior of the acids and sugars during growth and in cold storage is similar to the behavior of these constituents of some of the deciduous fruits—that is, it is apparently possible to remove the fruit from the tree after it is well along toward maturity and to ripen it in storage. The result will be an apparently sweeter fruit, due to loss of acidity and a reduced bitterness, the naringin or bitter principle breaking down in storage. A period in cold storage, then, renders the fruit more palatable. From the experiments detailed above it seems probable that the pitting of grapefruit can be controlled by curing at 70° F. before they are placed in cold storage. Investigations are in progress at the present time on this last-mentioned phase of the work.

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# ABSORPTION OF COPPER FROM THE SOIL BY POTATO PLANTS

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## RESULTS OF PREVIOUS INVESTIGATIONS

Some of the results obtained by a few investigators on the absorption of copper by plants and cells may be summarized as follows:

Schander[1] found that copper in a soluble form is a poison for plant cells of both high and low order.

Tschirch[2] believes that living plants are able to absorb copper through their roots and also through the epidermis of the leaves, the amount of copper absorbed being very small, however.

Haselhoff[3] stated that soluble copper salts are injurious to plants at a concentration of 10 mgm. of cupric oxid per liter. When soluble copper salts are added to the soil the plant materials, especially the potash and the lime, are dissolved and washed away, as a consequence of which the fertility of the soil is decreased. The action of copper sulphate is more severe on some crops than on others. The presence of calcium carbonate in the soil prevents or decreases the toxicity of solutions of copper sulphate.

True and Gies[4] have shown that when lime is used with copper sulphate solutions the toxicity of the copper is decreased. They state that when there is lime in the soil four times the amount of copper that can be allowed when no lime is found may be present in a soil without exerting a toxic action.

Forbes[5] found that corn grown in soil containing copper held most of the copper in the roots rather than in the tops. He states also that the toxicity of copper depends on the combination in which it exists in the soil, the physical characteristics of the soil, and the chemical composition of the soil, and on climatic and moisture conditions, as well as on the crop grown.

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## OBJECT OF PRESENT EXPERIMENTS

The experiments discussed in this paper were undertaken to determine what proportion of the copper present in standard Bordeaux spray, in Pickering's limewater Bordeaux spray, and in a solution of copper sulphate, of equal copper content, is absorbed by potato plants when the sprays or solution are applied directly to the soil in which the vines are growing. The comparative distribution of the absorbed copper in different parts of the potato plants was also studied.

The copper in the Pickering spray was in an insoluble form, basic copper sulphate, with no excess of lime present. The copper of the Bordeaux spray was in an insoluble form, with a large excess of lime present. The copper of the solution of copper sulphate was soluble. It was believed that a comparative study of these three sprays, containing copper in equal amounts, would show the extent to which the excess lime of Bordeaux spray is instrumental in preventing the absorption of copper by the roots of the potato plants, as well as the relation of the absorption of copper from a soluble copper compound to that from an insoluble copper compound when applied to the soil.

## EXPERIMENTAL WORK

The tests were conducted on the Aroostook Farm of the Maine Agricultural Experiment Station, at Presque Isle, Me., on Caribou type soil. A single row, 8 feet long, of Norcross strain of the Green Mountain variety of Irish potato plants was used for each of four plots which were treated in the following manner: Plot 1, sprayed with standard Bordeaux, 3–3–50 formula, containing 0.75 per cent of copper sulphate; plot 2, sprayed with an "A" formula Pickering limewater Bordeaux spray, containing 0.70 per cent of copper sulphate; plot 3, sprayed with a solution containing 0.75 per cent of copper sulphate; and plot 4, a control plot, unsprayed.

The vines were 20 inches above ground when the first applications were made. At each application 1 gallon of the spray or solution was applied directly to the ground within 6 inches of the stems of eight potato plants in each plot, each vine thus receiving 1 pint of the solution to each treatment. An equal amount of water was applied to the roots of eight control plants at the time the other applications were made. Applications were made on July 27, August 8, August 17, August 24, and August 30, 1917.

## PREPARATION OF SAMPLES

Vines and tubers from each of the four plots were taken for analysis at frequent intervals.

The vines from the various plots were dried in the air, then washed in running water and held for 30 seconds in a 4 per cent solution of hydrochloric acid, after which they were immediately washed in water and

finally in distilled water. The vines were next dried for 16 hours in an oven at 110° C. Separate analyses of leaves, stems, roots, and tubers were made.

Five or six tubers from each plot were thoroughly washed, rinsed in distilled water, and dried with a towel. The tubers were pared, passed through a grinder, well mixed, and transferred to a Mason jar with rubber and top. Care is necessary in securing a uniform sample of the ground tubers for analyses, as the water and solids separate very rapidly.

Samples of soil were taken 6 inches deep, near the roots of the treated plants, from the various plots at the time the plants were sampled. The soil samples were held in Mason jars with rubbers and tops until analyzed. Before analysis the stones and other foreign matter were removed from the samples.

## DETERMINATION OF COPPER IN VINES AND TUBERS

From 5 to 10 gm. of the dried leaves and stems, and from 1 to 5 gm. of the roots were taken for copper analyses. The samples were ashed in 4-inch porcelain dishes, 30 cc. of 5 per cent nitri acid were added, and the whole was allowed to remain overnight. The solutions were filtered and washed, after which ammonia was added to faint alkalinity. They were brought to a boil, cooled, and made to volume, usually 150 cc. The precipitated iron and alumina were removed by filtration, and an aliquot of the filtrate was taken for the determination of copper.

TABLE 1.—*Copper found in potato vines and tubers*[a]

| Plants taken for analysis. | Parts analyzed. | Soil treated with Pickering spray (0.75 per cent $CuSO_4$). | Soil treated with Bordeaux spray (0.75 per cent $CuSO_4$). | Soil treated with $CuSO_4$ solution (0.75 per cent $CuSO_4$). | Control plot. |
|---|---|---|---|---|---|
| | | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| Aug. 8 | Leaves | 0.0045 | ........ | 0.0065 | 0.0030 |
| | Stem | .0015 | 0.0070 | .0079 | .0043 |
| | Root | 0 | .0136 | .0052 | .0017 |
| 17 | Leaves | .0097 | .0100 | .0069 | .0089 |
| | Stem | .0023 | .0042 | .0047 | .0026 |
| | Root | .0036 | .0030 | .0101 | .0020 |
| | Tubers | .0004 | ........ | .0001 | .0001 |
| 24 | Leaves | .0179 | .0258 | .0109 | .0087 |
| | Stem | .0029 | .0048 | .0067 | .0022 |
| | Root | .0128 | .0104 | .0130 | .0010 |
| | Tubers | .0001 | .0001 | .0001 | .0002 |
| Sept. 3 | Leaves | ........ | ........ | ........ | ........ |
| | Stem | .0053 | .0069 | .0225 | .0017 |
| | Root | .0160 | .0069 | .0300 | 0 |
| Average | Leaves | .0107 | .0179 | .0081 | .0069 |
| | Stem | .0030 | .0057 | .0104 | .0027 |
| | Root | .0081 | .0085 | .0146 | .0012 |
| | Tubers | .0002 | .0001 | .0001 | .0002 |

[a] Analyses made on dry basis.

As a rule, 25-cc. aliquots were evaporated to dryness in 50-cc. po celain dishes on the steam bath, and the residue was taken up in 5 c of distilled water. Two drops of acetic acid and 3 drops of 1 per ce solution of potassium ferrocyanid were added, and the color was in mediately compared with that of standard solutions of copper sulpha which had been evaporated with ammonium nitrate and taken up 5 cc. of distilled water.

Copper in the ground tubers was determined by the same procedu using 50 gm. of the moist sample. The analytical data are recorded Table I.

DETERMINATION OF COPPER IN SOILS

One hundred gm. of the well-mixed soil samples were treated with mixture of 80 cc. of nitric acid and 20 cc. of sulphuric acid in large po celain casseroles. The mixtures were heated on the steam bath a then on the hot plate until the nitric acid fumes were removed. T residues were extracted with 200 cc. of water, filtered, washed, and ma to 500 cc. volume. After evaporation to 200 cc., the iron was preci tated with ammonia and the solutions were made to volume. They we next filtered and aliquots were made acid with hydrochloric acid, throu which hydrogen sulphid was passed for 20 minutes, or until all the copp was precipitated. The precipitated copper after settling was filter and dissolved in 10 cc. of nitric acid, the filter paper and precipitate bei transferred together. Ammonia was added to faint alkalinity, and t solutions were evaporated to dryness in small porcelain dishes. The re dues were taken up in 5 cc. of distilled water, two drops of acetic ac and three drops of 1 per cent potassium ferrocyanid were added, and t copper was estimated by colorimetric comparisons. In some cases aft evaporation to dryness it was necessary to take up in water, filter, was and repeat the evaporation to remove precipitated material.

If present in large enough amounts copper may be determined elect lytically, by a method based on the procedure given by Forbes, Free, a Ross.[1]

The results of the analyses of the first and last samples of soil tak appear in Table II. This table gives also the results of a series of te on the soil around the roots of potato plants which had been comme cially sprayed with Bordeaux, with Pickering spray, and with a soluti of copper sulphate, to determine whether any appreciable amounts of t copper occur in the soil beneath the sprayed vines.

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TABLE II.—*Copper found in soil*

SPRAYS APPLIED TO SOIL NEAR PLANT ROOTS

| Sample No. | Date of sampling | Description of sample | Description of plots. | Total copper found in soil. |
|---|---|---|---|---|
| | | | | *P. p. m.* |
| 1 | July 26 | Samples taken before any copper was added to the soils. | Control | 2 |
| 2 | | | Pickering "A" formula | 1 |
| 3 | | | Bordeaux 3-3-50 | 2 |
| 4 | | | $CuSO_4$ solution | 2 |
| 5 | Aug. 24 | Samples taken just before last application of copper to soil. | Control | 5 |
| 6 | | | Pickering "A" formula | 211 |
| 7 | | | Bordeaux 3-3-50 | 256 |
| 8 | | | $CuSO_4$ solution | 250 |
| 9 | Sept. 3 | Samples taken after the last application of copper to the soil. | Control | 2 |
| 10 | | | Pickering "A" formula | 225 |
| 11 | | | Bordeaux 3-3-50 | 243 |
| 12 | | | $CuSO_4$ solution | 449 |

SPRAYS APPLIED TO VINES IN COMMERCIAL PRACTICE

| Sample No. | Date of sampling | Description of sample | Description of plots. | Total copper found in soil. |
|---|---|---|---|---|
| A | July 16 | Samples taken before any sprayings were made in 1917. | Control | 1 |
| B | | | Pickering "C" formula | 1 |
| C | | | Bordeaux 5-5-50 | 1 |
| D | | | Pickering "A" formula | 1 |
| E | Aug. 31 | Samples taken after last sprayings were made in 1917. | Control | 2 |
| F | | | Pickering "C" formula | 2 |
| G | | | Bordeaux 5-5-50 | 2 |
| H | | | Pickering "A" formula | 3 |

## DISCUSSION OF RESULTS

### VINES AND TUBERS

The leaves, stems, and roots of the plants from the soil receiving the Pickering spray showed an increased copper content with each successive analysis (Table I). The largest percentage of the copper was held by the leaves. The roots held an appreciable part of the copper, the amount increasing from 0 in the first sample to 0.0160 per cent in the sample taken on September 3. The tubers contained only minute amounts of copper.

The plants from the Bordeaux treated soil showed irregularities, particularly with respect to the copper content of the roots and stems. The leaves and stems contained more copper than those of the plants from the Pickering treated soil, while the roots contained less copper than the roots of the plants from the Pickering treated soil. The amounts of copper found in the tubers were small.

The vines grown in the soil treated with a solution of copper sulphate showed a marked progressive increase in copper content of the roots with each succeeding analysis. The leaves contained somewhat larger

amounts of copper than the stems, but not as much as the roots. The leaves contained less copper than the leaves of the plants grown on the Bordeaux or Pickering treated soils. The tubers from the plot treated with copper sulphate solution were as low in copper as those from the other plots.

The analyses of the various portions of the control plants showed the presence of copper, but in smaller amounts than in the plants grown on soil treated with the copper sprays.

The results of the copper absorption experiments indicate that the potato plants, with the exception of those grown in the soil receiving the solution of copper sulphate where the roots were distinctly injured, distributed the largest part of the absorbed copper to the leaves, while the roots and stems contained appreciable amounts of copper. In all normally sprayed potato plants the largest proportion of the copper is said to be found in the leaves.

The plants grown on the soil treated with a solution of copper sulphate were small and lacking in vigor. The roots had but few hairs, and showed other signs of injury. The large percentage of copper found in the roots, together with the small size of the roots, indicated some interference with the normal metabolism of the vines. The toxic effect of the soluble copper salt was exerted primarily on the roots of the plants. It was apparent that the soluble copper sulphate had injured the potato plants, while the insoluble copper compounds had not.

The vines from the Bordeaux plot contained a little more copper than the vines from the Pickering plot, indicating that the extra lime of the Bordeaux spray did not aid in preventing the absorption of copper by the plants.

SOIL

The results of the analyses of the first and last samples of soil taken show that no water-soluble copper was found in any of the samples examined. The amount of copper in the first set of samples (Table II, No. 1, 2, 3, and 4) which were taken before any copper had been added to the soils, is practically the same in all cases. The sets of samples taken before and after the final treatment of the plots show the presence of a large amount of copper in the samples receiving the copper treatments. This means that copper in an insoluble form may be present in the soil in marked amounts without exerting any apparent toxic action on the growth of potato plants.

But little copper was found in the soil as a result of spraying with copper sprays according to commercial practice.

On September 5, shortly after the last treatment of the soil, a few hills of potatoes were dug. The weights and number of the tubers, the percentage of decayed tubers, as well as the notes taken on the size and appearance of the vines are given in Table III.

TABLE III.—*Effect on potato tubers and vines of applications of sprays to soil*

| Spray used. | Tubers.[a] | | | Stand and condition vines. |
|---|---|---|---|---|
| | Number and size. | Weight | | |
| | | Total. | Average. | |
| | | *Ounces.* | *Ounces.* | |
| Bordeaux (0.75 per cent $CuSO_4$). | 3 (1 large and 2 small) in 1 hill. | 8 | $2\frac{2}{3}$ | Normal. |
| Pickering (0.7 per cent $CuSO_4$). | 8 (5 large and 3 small) in 2 hills. | 20 | $3\frac{5}{8}$ | Do. |
| Copper-sulphate solution (0.75 per cent $CuSO_4$). | 17 (all small) in 4 hills. | 23 | $1\frac{1}{2}$ | Small and stunted. |
| Unsprayed (control). | 8 (3 large, 3 medium, and 2 rotten) in 2 hills. | 24 | 3 | Normal stand; blight. |

[a] Rot found only on control tubers.

These data show that the solution of copper sulphate had a very disastrous effect on the growth and yield of the tubers. The only decayed tubers found were obtained from the unsprayed plot. These results are so few that they can be considered only as suggestive.


## SUMMARY

Potato plants grown in soil treated with insoluble copper compounds contained more copper in the leaves than in the stems, while but little copper was found in the roots. The tubers showed only traces of copper.

When the soil was treated with the copper sulphate solution, the roots were injured and the normal metabolism of the vines was disturbed. The tubers from these vines were small and the vines stunted. The roots of these plants held more copper than the leaves.

The soluble copper sulphate added directly to the soil caused injury to the plants, while the insoluble copper compounds of the sprays did not. The excess lime of the Bordeaux spray did not reduce the amount of copper absorbed by the plants compared with the plants grown on the Pickering plot.

Practically the same amounts of copper were found in all the soil samples tested. Samples of soil from sprayed potato fields showed but minute amounts of copper.

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## PALE WESTERN CUTWORM (POROSAGROTIS ORTHOGONIA MORR.)

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### INTRODUCTION

Extensive injury to grain crops by cutworms was reported from north central Montana during the period from 1915 to 1920. It was at first supposed that well-known species were responsible for the damage and the usual method of control, poisoned bran mash, was recommended. The repeated failure of this method led to a study of the situation, and from the results of numerous rearing records and the personal investigation of many infested fields it was found that the greater part of the losses was caused by the pale western cutworm (*Porosagrotis orthogonia* Morr.), a species previously not considered of economic importance in Montana. The enormous damage which it has done during the last six years, the rapidity with which it has extended its range, its unusually long period of larval feeding, its comparative freedom from parasites, and the fact that it works underground and can not be controlled by poisoned bran mash, stamp *P. orthogonia* as the most dangerous of all our western grain cutworms, not excepting even the army cutworm (*Chorizagrotis auxiliaris* Grote).

### HISTORY OF THE SPECIES

The species was given its specific name in 1876 by Morrison (*15*),[2] who described it as *Agrotis orthogonia* from specimens collected at Glencoe, Nebr. In 1890 the species was placed under the genus Porosagrotis by Smith (*16, p. 129*), who also gave a description of the adult and recorded its occurrence in the following new localities: Colorado, New Mexico, Arizona, and Utah. Dyar (*3, p. 139*) lists the species and gives its range as the Rocky Mountain region. In 1905 it was reported

[1] The color plate and drawings for this article were done by Miss Helen Lund, with the remarkable accuracy characteristic of her work. Mr. K. M. King, an undergraduate assistant in 1919, conducted the rearing experiments during that year, and many of the observations recorded are based on his very complete insectary notes.

[2] Reference is made by number (italic) to "Literature cited," p. 320–321.

(doubtfully) by Dod (*2, v. 37, p. 53*) from Calgary, and in 1908 by Hampson (*9, p. 102*) from Prairie, Alberta.

The species was looked upon as a rare insect until 1911, when Gibson (*5, 6*) reported it under the name *Porosagrotis delorata* Smith as destroying large areas of wheat in southern Alberta, where one correspondent claimed to have lost 320 acres before June 21. Hewitt (*10, p. 177*) also refers to this outbreak in his annual report for 1912, and in his report (*11, p. 506*) for the following year the species is recorded along with *Euxoa ochragaster* Gn. as having destroyed between 30,000 and 35,000 acres of crop in 1912 in about the same territory where damage occurred the previous year.

An account of the insect's first appearance, its depredations during 1911 and 1912, and a brief review of the control experiments carried on the following year was given by Gibson (*7*) in 1914. In this article he states that *Porosagrotis delorata* Smith and *P. orthogonia* Morr. are the same and records an adult of the species as having been taken at Regina, Saskatchewan, on August 10, 1904. Hewitt (*12, p. 861–862*) states that in 1913 this cutworm caused much less damage to crops in southern Alberta than in the preceding year.

The most complete published account of the species was written by Gibson (*8, p. 30–31*) in 1915. A brief description of the larva and adult is given, together with notes on its life history and habits. In this article the common name "pale western cutworm" is used, apparently for the first time.

During the season of 1914 *Porosagrotis orthogonia* was again present in Alberta, and experiments in its control were conducted by Strickland (*17*), who found that surface applications of the bran mash were wasted but that gratifying results were secured when a molasses-and-shorts mixture was harrowed into the soil. The next account is by the same author (*18*), who gives a brief statement of the life history and makes recommendations for the control of the species by cultivation methods and the modified use of a poisoned-shorts mixture.

According to Hewitt's 1916 report (*13*), the pale western cutworm was seldom seen in 1915. Experiments, however, were conducted by Strickland which confirmed his earlier conclusions that shorts is preferable to bran and that when the soil is moist harrowing in the poison is not so advantageous as it is on dry soil.

In his 1919 annual report (*1, p. 8*) Cooley points out the habits of the species which make it such an important pest and places its control as one of the most important entomological problems in Montana.

A review of the life history of the species, descriptions of the various stages, and colored drawings of egg, larva, and adult, are given by Maxson (*14, p. 45–46*) in his work on sugar-beet insects published in 1920.

## DISTRIBUTION

Published records of the occurrence of *Porosagrotis orthogonia* are as follows: Glencoe, Nebr., by Morrison (*15, p. 239*); Colorado, New Mexico, Arizona, and Utah by Smith (*16, p. 129*); Rocky Mountains by Dyar (*3, p. 139*); Calgary, Alberta. (doubtfully) by Dod (*2, 37, p. 53*); Prairie, Alberta, by Hampson (*9, p. 102*); and southern Alberta by Hewitt (*10, p. 177*), Gibson (7) and Strickland (*17*).

In Montana *Porosagrotis orthogonia* now occurs throughout the State east of the continental divide. It has been most abundant in the tier of counties which lies just east of the foothills of the main range of the Rocky Mountains and which extends from the Canadian border to within 100 miles of the southern border of the State.

Mr. E. H. Strickland has kindly given the following information on the present distribution of *Porosagrotis orthogonia* in Canada:

> Our records indicate that it is practically confined to southern Alberta, extending as far north as latitude 51° and east to longitude 108°, although it has been recorded as far as Regina, Saskatchewan. The maximum intensity, however, is confined to an area that does not extend more than 100 miles east of the Rocky Mountains

Dr. William Barnes, of Decatur, Ill., who has an extensive collection of western noctuids, has generously furnished the following records of *Porosagrotis orthogonia* specimens in his collections: Denver, Oak Creek Canyon, Lavetta, and Alamosa, Colo.; Deming and Fort Wingate, N. Mex.; Provo, Vineyard, and Eureka, Utah; Yellowstone National Park, Wyo.; Reno, Nev.; Redington, Ariz.; and Kern County, Calif.

Mr. George M. List states that *Porosagrotis orthogonia* is fairly common at Fort Collins, Colo., 430 moths having been taken at a trap during the season of 1920.

To Mr. C. N. Ainslie, of the Bureau of Entomology, United States Department of Agriculture, we are indebted for a record of 63 *Porosagrotis orthogonia* moths reared from a shipment of larvæ from Dickinson, N. Dak., on June 10, 1920.

From the foregoing records it is evident that *Porosagrotis orthogonia* occurs at least in scattering numbers throughout the southwestern and northwestern States with the possible exceptions of Oregon, Washington, and Idaho, where as yet it has not been collected. Correspondence with entomologists throughout the territory where *P. orthogonia* has been recorded indicates that it has never been of economic importance outside of the heavily infested areas in Montana and Canada.

## METHODS OF STUDYING

### IN THE INSECTARY

Larvæ were reared in individual tin boxes 1.5 inches in diameter. The bottom of each box was covered with filter paper which was slightly moistened at each feeding. This prolonged the freshness of the wheat

and dandelion which were used as food, and the cans could be easily cleaned by replacing the filter paper whenever it became soiled.

Pupæ were placed in moist, well-pulverized soil in individual glass vials 1 inch in diameter and 4 inches deep. Each vial was filled to a depth of 2 inches with well-firmed soil in which a round hole 1 inch deep was punched to receive the pupa, which was placed in it with the anterior end uppermost. A small twig was placed in each vial so that the moth upon emerging could hang from it and allow the wings to expand. The vials were closed by cheesecloth held in place by rubber bands. The best results were obtained by keeping the pupæ in uniformly moist and mellow dirt. Extreme dryness or excessive moisture often resulted in the death of the pupæ. Uniform moisture conditions were more easily obtained by allowing water to run slowly down the side of the tube instead of flooding it over the surface of the soil.

After the moths emerged they were placed in wire screen covered tin cans 3.5 inches in diameter and 2.5 inches deep. An inch of moist soil was kept in the bottom of the cans, and alfalfa or clover blossoms were added each day for the moths to feed upon (Pl. 30, A) and to hide under. Cutworm moths of all kinds seem very contented in these cans, and with *Porosagrotis orthogonia* no difficulty was encountered in getting the females to mate and lay eggs.

Eggs were placed on filter paper in pint Mason jars with the caps lightly screwed down. A few drops of water were placed on the filter paper from time to time to provide the proper amount of humidity.

### IN THE FIELD

A very good opportunity to watch the development of this insect under natural conditions was afforded in a heavily infested field at Wilsall, Mont., in 1919. This field was first examined on May 1 and was visited several times a month all summer. Many fields in other parts of the State were also visited, but that at Wilsall was the only one where *Porosagrotis orthogonia* was followed through all stages of its development. During the summer of 1920 a temporary field station was established at Willow Creek, Mont., in a district where thousands of acres of wheat had been destroyed during May and June. Moths appeared in large numbers during August and September and were under observation at all hours, both day and night. Special attention was given to the egg-laying habits, and for this purpose two observation cages were set up. The cages were 2-foot cubes with screen wire sides and solid metal tops. They were placed over sunflower plants, and the ground inside the cages was covered with soft dirt, stubble, clods, baked earth, and green plants, thus offering the moths nearly all the natural conditions of the neighborhood. Experiments with trap lights were also carried on at the Willow Creek field station.

## SEASONAL HISTORY AND HABITS

### DURATION OF EGG-LAYING PERIOD

During the season of 1919 the eggs were found to be well developed in the ovaries when the female moth first emerged, and egg laying began and was completed within a short time under insectary conditions. The first eggs were obtained from reared moths on August 17, the average period between emergence and the beginning of egg laying being four days. Moths collected in the field on August 26, which appeared to have just emerged, laid numerous eggs the following day and continued to lay until September 9.

In 1920 no records were available from reared moths, but a study of moths in the field seemed to indicate that the eggs that season were not fully developed when the moths first emerged. Thus out of 35 moths examined on August 24 only one had well-developed eggs in the ovaries. On September 1 many moths were found with the ovaries filled with well-developed eggs. These moths were mostly badly rubbed specimens, indicating that they had probably emerged some little time before.

Our field observations show that the height of the egg-laying period is during the last week in August and the first week in September. Eggs in smaller numbers may be laid during the first three weeks in August and as late as October 1.

### WHERE EGGS ARE LAID

The first eggs obtained were from moths confined in tin rearing cans. When the soil in the cans was dry and light most of the eggs were placed from ¼ inch to 1 inch below the surface and could be found only by careful searching. When the soil was hard and lumpy eggs were scattered about on the surface and could be easily seen. In the rearing cans the eggs were not always laid in the soil. Many of them were placed on the stems, leaves, and flowers of alfalfa, and frequently scattering eggs were found on the sides of the cans or on the screen covers. Thus out of 243 eggs found in one can, 180 were found in the soil, 62 were found on the stems, leaves, and flowers of alfalfa, and 1 was found on the side of the can. Some were laid singly and others were in clumps of 2, 3, or 4, and sometimes as many as 40.

In outdoor cages, where a variety of soil conditions and various kinds of vegetation were available, eggs were laid only in loose, dry dirt.

Under field conditions the eggs are very difficult to find, and the only ones we have ever found were secured by carefully examining the soil at the exact spots where females were seen in the act of egg laying. Eggs are found most frequently in loose, mellow dirt from ¼ to ½ inch below the surface. This is an important point, and will be discussed later in connection with the habits of the moths and from the standpoint of control methods.

## NUMBER OF EGGS

The only females upon which we have complete records are five moths reared and mated in the insectary. They averaged 315 eggs, the lowest number per moth being 248 and the highest 453. Ten moths brought in from the field averaged 132 eggs, but these had probably laid numerous eggs before they were caught. Under normal field conditions the average number per female is probably between 300 and 400. The records of the individual moths are shown in Tables I and II.

TABLE I.—*Time between emergence and egg laying, length of egg-laying period, and number of eggs laid by reared specimens of Porosagrotis orthogonia*

| Moth No. | August, 1919. | | | | | | | | | | | | | | | | | | | Eggs in ovaries at death. | Total number eggs. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | | |
| 1 | E | | | | | 243 | D5 | | | | | | | | | | | | | 0 | 248 |
| 2 | E | | | | 9 | D | | | | | | | | | | | | | | 249 | 258 |
| 3 | | | | | | E | | 88 | 45 | 96 | 54 | D | | | | | | | | 0 | 283 |
| 4 | | | | | | E | | | | 60 | 251 | 25 | 26 | 75 | D | | | | | 0 | 453 |
| 5 | | | | | | E | | | | 305 | 2 | 12 | | | 12 | | | | | 2 | 333 |

E=emerged. D=dead.
Average period between emergence and egg laying ........ 4 days.
Average period of egg laying ........ 3 to 4 days.
Average number of eggs ........ 315.

TABLE II.—*Period of egg laying and number of eggs laid by Porosagrotis orthogonia moths brought in from the field and kept in rearing boxes*

| Moth No. | August | | | | | | September. | | | | | | | Eggs in ovaries at death. | Total number eggs. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 26 | 27 | 28 | 29 | 30 | 31 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| 1 | Caught | | | | | 63 | D 100 | | | | | | | 4 | 167 |
| 2 | do | | | | | | D 23 | | | | | | | 91 | 116 |
| 3 | do | 22 | 74 | 43 | | | 45 | D | | | | | | 0 | 184 |
| 4 | do | 2 | 5 | 3 | 4 | | 2 | D 3 | | | | | | 88 | 107 |
| 5 | do | | | | | | 6 | D 3 | | | | | | 103 | 112 |
| 6 | do | | 4 | 56 | 25 | | 15 | D 14 | | | | | | 20 | 134 |
| 7 | do | 20 | 35 | 15 | 30 | 12 | 6 | D 8 | | | | | | 1 | 127 |
| 8 | do | | 26 | 20 | 32 | 22 | 16 | | D | | | | | 0 | 116 |
| 9 | do | | | | | | | 36 | 44 | D 26 | | | | 0 | 106 |
| 10 | do | | | | | | | | | | D | | | 152 | 152 |

D=dead.
Average period of egg laying ........ 3 to 4 days.
Average number of eggs ........ 132.

## DURATION OF EGG STAGE

The length of the egg stage is exceedingly variable, depending largely upon moisture conditions. It may be as short as 11 days or may extend over several months. Eggs laid in the insectary August 19 hatched August 30. On September 30 several newly hatched larvæ were found

at Wilsall. Hundreds of eggs were laid in the breeding cans during the last week in August and the first week in September, but with the exception of three larvæ which hatched August 30 no eggs hatched unless they were placed in a very humid atmosphere. The larvæ mature within the eggs in from 10 to 20 days and may remain for months in this condition, waiting merely for proper moisture conditions to allow them to break through the eggshell. Examples of this may be of interest. Moth number 1427–G laid 160 eggs on August 24 and 25. These were placed on a piece of filter paper in a Mason jar. They were allowed to stand in the laboratory for 10 days and were then placed in an incubator and held at day temperature of 80° F. and night temperature of 60°. At the end of three weeks practically all the eggs had darkened, and the black heads of the young cutworms could be seen through the eggshells, but none had hatched. On October 4 a few drops of water were added to the filter paper, and when the jar was opened 24 hours later 70 larvæ were found to have hatched. On October 11 water was again added to this jar, and 40 more larvæ hatched out.

On October 27 a few drops of water were added to another jar of 62 eggs which were laid August 29. In two hours 8 larvæ had hatched, but no more hatched during the next six hours. Twenty-four hours later all of the eggs had hatched. On October 27 moisture was added to eggs that had been kept in the greenhouse since August 30, and in two hours many of them had hatched. On this same date one of these eggs was placed on a block of plaster of Paris, and water was slowly dropped upon it from a medicine dropper. At the second drop the larva began to move within the egg. Soon it began to move its mandibles and after several attempts the eggshell was punctured, and within 30 minutes after the first drop of water was added the larva was free from the shell and actively moving about. On November 20 eggs that were laid August 30 and had been kept for a month in a small tin can on a shelf directly over a radiator were examined, and living larvæ were removed from them by carefully breaking the eggshells with fine needles.

On November 1 twenty eggs which were laid on August 29 and had been kept indoors were placed in two small wooden boxes and buried in a pail of damp sand. The pail was set on the ground, outdoors, where it was covered with snow practically all winter. The eggs were examined once a month, but none hatched until the second week in April when all the eggs were found to have hatched and the larvæ were alive and vigorous.

From our studies of the egg it would appear that if there is sufficient moisture and proper temperature condition the majority of the eggs will hatch in the fall, while if it is unusually dry or cold weather starts early the eggs will not hatch until the following spring. Strickland (*18*) found eggs on frozen ground December 3, which would indicate that in Canada some of the eggs at least do not hatch until spring.

## FIRST APPEARANCE OF LARVÆ

Larvæ may appear in the fall. This is proved by the fact that three larvæ were found at Wilsall on September 30 and that larvæ hatched in the rearing boxes during October and November whenever sufficient moisture was added. Large numbers of larvæ must have hatched at Wilsall during the fall of 1918, for as soon as the snow left the ground the following spring fourth- and fifth- instar larvæ were found in large numbers. We have no records of injury to wheat during the fall months, but it seems quite probable that in years when there is considerable moisture and mild weather during October and November great damage may be done.

The larvæ begin to feed shortly after the wheat begins to grow in the spring. In 1919 at Wilsall 80 acres of winter wheat were completely destroyed by May 1, which indicates that the worms must have been active 10 days or 2 weeks previous to that date.

In 1921 at Willow Creek first-instar larvæ were found on March 3. The weather had been warm for about a week, and winter wheat was starting to grow again. No larger larvæ could be found, and it seemed as though the very small first-instar larvæ must have just hatched.

## PERIOD OF LARVAL FEEDING

One of the reasons why *Porosagrotis orthogonia* is such a dangerous insect is the unusually long period of heavy larval feeding which extends until the middle of June or even to July 10 in the case of late-hatched specimens. Judging from the reports of injury received, the larvæ attract most attention during the month of May and the first two weeks in June, differing decidedly in this respect from *Chorizagrotis auxiliaris*, which generally has reached the height of its destructiveness by April 15 and has practically disappeared by May 1.

The length of the larval stage as determined for 20 larvæ, 5 from each of 4 parent moths, under insectary conditions varied from 62 to 151 days and averaged 118 days, as shown in Table III.

All of the larvæ were kept under very similar conditions, and no reason has been found for the wide variation. They were always fed at the same time and were kept on one tray in individual rearing boxes, thus giving practically identical conditions of moisture, temperature, and food. In spite of this similarity of conditions we find that larva 21e pupated 62 days after hatching from the egg, while larva 21d from the same parent and from the same egg cluster took 124 days, or just twice as long, to reach the same stage of development. The number of instars was also found to vary. Thus the number of larval instars for the five larvæ from each of the moths was as follows: Moth 21—four had 7 instars and one had 8; moth D—all five passed through 8 instars; moth 42—two had 7 instars and three had 8; moth 24—one had 7 instars and

four had 8. According to the rearing records of other larvæ than those shown in the table, several individuals passed through 9 larval instars and one passed through 10. Eight instars, however, is the usual number and the minimum is 7.

TABLE III. *Duration of larval instars of Porosagrotis orthogonia under insectary conditions*

| Larva record No. | Number of days in each instar | | | | | | | | Number of days in larval stage. |
|---|---|---|---|---|---|---|---|---|---|
| | I. | II. | III. | IV. | V. | VI. | VII. | VIII. | |
| 21a | 9 | 5 | 8 | 8 | 12 | 13 | 44 | ..... | 99 |
| 21b | 11 | 6 | 7 | 9 | 10 | 13 | 29 | ...... | 85 |
| 21c | 12 | 6 | 8 | 8 | 14 | 18 | 32 | ...... | 98 |
| 21d | 12 | 9 | 11 | 9 | 9 | 13 | 16 | 45 | 124 |
| 21e | 9 | 7 | 8 | 7 | 11 | 17 | 14 | ...... | 62 |
| Da | 12 | 9 | 10 | 9 | 11 | 13 | 14 | 37 | 125 |
| Db | 13 | 9 | 10 | 7 | 11 | 9 | 14 | 48 | 121 |
| Dc | 12 | 10 | 7 | 13 | 9 | 16 | 21 | 63 | 151 |
| Dd | 12 | 7 | 9 | 10 | 10 | 16 | 16 | 45 | 125 |
| De | 13 | 9 | 12 | 14 | 17 | 15 | 19 | 51 | 150 |
| 42a | 11 | 8 | 12 | 10 | 11 | 14 | 25 | 32 | 123 |
| 42b | 10 | 5 | 9 | 10 | 9 | 20 | 41 | ...... | 104 |
| 42c | 14 | 10 | 10 | 9 | 18 | 17 | 34 | ...... | 112 |
| 42d | 12 | 8 | 11 | 10 | 12 | 12 | 14 | 33 | 112 |
| 42e | 13 | 11 | 7 | 10 | 12 | 13 | 20 | 33 | 119 |
| Ma | 9 | 6 | 8 | 10 | 10 | 9 | 21 | 75 | 148 |
| Mb | 7 | 7 | 10 | 15 | 13 | 18 | 18 | 56 | 144 |
| Mc | 13 | 9 | 9 | 9 | 9 | 16 | 18 | 65 | 148 |
| Md | 13 | 12 | 12 | 10 | 9 | 14 | 18 | 43 | 131 |
| Me | 7 | 7 | 10 | 10 | 10 | 13 | 26 | ..... | 83 |
| Average | 11.2 | 8 | 9.4 | 9.8 | 11.3 | 14.4 | 22.6 | 29.6 | 118 |

FEEDING HABITS OF LARVA

The larva differs from that of most cutworms in its feeding habit in that it almost invariably attacks the plant below the surface of the ground. The most frequent type of injury is the eating away of the central stem and its surrounding sheaths from ½ inch to 1 inch below the surface of the ground. In many instances the stem is severed and the lower part not eaten, and frequently it is only slightly chewed into before the worm moves on to another plant. Even slight injury to the underground portion of the central stem usually results in the death of the plant. A very small portion of each plant is actually eaten by this cutworm, and its capacity for destruction is thus greatly increased. The first indication of injury is the presence of wilting or dried plants which can be easily lifted out of the soil without bringing the roots. The destruction of grain that is just pushing through the ground is particularly rapid, one worm being able to cut off plant after plant in quick succession. The worm usually moves along the drill row, taking each plant as it goes (Pl. 30, D). Where one crop has been destroyed and

the land has been reseeded the worms often attack sprouting grain and sometimes even gnaw into the kernels before they germinate.

The larvæ feed during both day and night. Freshly cut plants have been found repeatedly during the day and cutworms have been found with their heads inside the sheath of the plant in the act of feeding on the central stem. In the rearing boxes no difference could be noticed between the amount of day and of night feeding.

According to observations made by Strickland and reported by Gibson (7) it is the habit of the larva—

> to travel over the surface of the soil and when a suitable plant for attack is found it immediately burrows and feeds just below the surface.

In our observations we have never witnessed this habit. Considerable time has been spent during the late afternoon and evening in heavily infested fields, and except in rare instances we have never seen *Porosagrotis orthogonia* larvæ above ground. On the other hand, we have closely examined hundreds of newly attacked plants where there was no sign that the soil had been disturbed at the surface by the burrowing of the larva. In order to find out whether the worms would work from one plant to another without coming to the surface, wheat seedlings were started 3 inches apart in a flat in the greenhouse, and when the wheat was up about 1 inch 12 half-grown cutworms were placed in one end of the flat. At the same time a line of plaster of Paris was placed across the center of the flat so that any traveling of the worms over the surface at the center would be indicated by lines through the plaster of Paris. The seedlings at the end of the flat where the worms were introduced were immediately attacked, and within a week all of the seedlings in the flat had been cut off below the surface of the soil and without any indication that any of the larvæ had crossed the plaster of Paris line.

If it were the natural habit of *Porosagrotis orthogonia* to travel over the surface of the ground in getting from plant to plant, it would come in contact with poisoned bran mash scattered on the ground, and it seems as if there would be no difficulty in controlling it by the ordinary methods. The fact that poisoned bran mash is useless against *Porosagrotis orthogonia*, together with our field and insectary observations, leads us to believe that it very rarely comes to the surface in getting from one plant to another but instead moves underground, generally along the drill row.

We have received occasional reports from farmers stating that pale western cutworms had been seen feeding above ground during and immediately following rains, but only one instance of this kind has come under our own observation. This was at Willow Creek on June 27, 1920. A light thunder shower at 6.30 p. m. cooled the air and wet the ground to the depth of 1 inch. As soon as the storm was over, numerous cutworms were seen crawling over the surface in a wheat field that

was known to be badly infested with *Porosagrotis orthogonia*. It was estimated that about 50 per cent of the total number of cutworms in the soil were on the surface at any one time. They appeared in greatest numbers in the spots where the grain had previously been cut off. At first they merely wandered about over the surface, but later on, as it became dark, they started feeding. Observations were continued until 11 p. m., at which time the worms were still moving about and feeding upon the leaves of wheat and grass. At 5 a. m. the next morning a few worms still remained on the surface, but all disappeared as the sun came up. The soil around stray wheat plants was noticeably stirred up where the worms had come up and gone down. That this habit of feeding above ground is not a common one is shown by the fact that only in rare instances have we ever found any injury to that part of the grain plant which is above the ground, and in such cases there was always some doubt as to whether the injury might not have been done by some other species.

### LARVÆ DO NOT LEAVE FIELDS AFTER GRAIN IS DESTROYED

Another unusual habit of *Porosagrotis orthogonia* is that it seldom migrates even though its food supply becomes exhausted. If, in following along the drill row, it fails to find a plant within a few feet, it simply remains where it is, perhaps for several weeks, without feeding or growing to any extent. In fields that are only partially infested the injury shows up as scattered bare spots, and in such places the larvæ do concentrate along the edges of the standing grain, but we have never known them to migrate more than a few rods. This habit of remaining in the fields where grain has been destroyed has a very important bearing on farm practice, as will be shown by the following example: Eighty acres of winter wheat at Wilsall in 1919 were completely destroyed by May 1, the ground being left entirely bare. The field was reseeded to spring wheat the second week in May. On May 24 the grain was just coming through the ground and was being rapidly cut off by cutworms which had remained in the field since the winter wheat had been destroyed, some three weeks before. The worms continued to feed for several weeks and destroyed all the spring wheat.

The ability to go for a long time without feeding was well shown by a half-grown larva which remained in a rearing can for 12 weeks without food and was then fed and reared to maturity.

### FOOD PLANTS

In Montana this cutworm has been most commonly found feeding upon winter and spring wheat. Oats, barley, rye, flax, and alfalfa have also been attacked. In the insectary, larvæ have fed readily and grown rapidly upon dandelion. In Canada, Gibson reports *Porosagrotis*

*orthogonia* larvæ as feeding upon fall and spring wheat, oats, barley, flax, beets, onions, cabbages and carrots.

PERIOD OF INACTIVITY BEFORE PUPATION

Although *Porosagrotis orthogonia* larvæ are mature and have practically ceased feeding by the middle of June they do not pupate until nearly a month later. During this period they occasionally feed slightly, but for the most part they remain in a semidormant condition, gradually turning whitish in color and shrinking in size just previous to pupation. This was noticed both in the field and under insectary conditions. Notes taken at Wilsall June 20, 1919, state that on that date cutworms were decreasing in numbers and were nearly all full grown. This field was visited again on July 4, when many whitish larvæ were found, some of which had formed earthen cells, but no pupæ were found in a two-hour search.

Records kept on 75 larvæ in the insectary showed an average period of 20 days of complete inactivity previous to pupation and a period of 26 days in which only very slight feeding took place.

PUPAL PERIOD

Pupation generally takes place about the middle of July. Out of 80 specimens collected as larvæ at Wilsall in May, 1919, and reared to adults in the insectary, the average date of pupation was July 19, the earliest date July 2 and the latest August 11. This checked out almost exactly with conditions in the field at Wilsall.

About a month is spent in the pupal stage. The average length of pupal period of 80 specimens was 29½ days, the shortest 21 days and the longest 40 days.

The pupæ are protected by a cemented earthen cell and are usually found at a depth of 3 to 4 inches in the soil beneath the plants where they last fed.

SEASONAL ABUNDANCE OF ADULTS

The earliest emergence of *Porosagrotis orthogonia* moths which we have on record is July 31, although Gibson (*8, p. 30–31*) reports the emergence of a moth of this species on July 19. In general, the period of greatest abundance is during the last two weeks in August and the first week in September.

The field at Wilsall where *Porosagrotis orthogonia* larvæ destroyed two seedings of wheat during May and June, 1919, was searched for moths on August 7, but none could be found. A trap light was run until midnight on this date, and not a moth of this species was taken. On August 26 the field was again visited, and numerous moths were found during the day, and at night they came to trap lights by the thousands. The majority of the moths taken at this time were in prime condition and

looked as though they had just emerged. One week later the number of moths was greatly reduced; they were difficult to find during the day, and very few came to lights at night. On September 30 an entire day was spent in searching the same field, but not a moth could be found. The owner of the field had been disking and drilling throughout the month of September and during the first half of the month had frequently seen moths fly up as the ground was disturbed but had seen none after September 15.

Seventy-five larvæ collected in this field May 1 and reared in the insectary at Bozeman emerged as adults on the dates shown in Table IV. In 1920 at Willow Creek the first moth was caught on August 9, the heaviest flight was from August 19 to 24, and several moths were seen as late as October 8.

TABLE IV.—*Dates of emergence of Porosagrotis orthogonia moths in 1919*

| Date. | Number of moths. | Date. | Number of moths. |
|---|---|---|---|
| July 31 | 1 | Aug. 17 | 4 |
| Aug. 4 | 3 | 18 | 13 |
| 5 | 1 | 19 | 3 |
| 6 | 1 | 20 | 2 |
| 7 | 2 | 22 | 5 |
| 8 | 2 | 23 | 5 |
| 9 | 1 | 24 | 4 |
| 10 | 2 | 25 | 4 |
| 12 | 1 | 26 | 2 |
| 13 | 1 | 27 | 1 |
| 14 | 4 | 28 | 1 |
| 15 | 3 | 31 | 1 |
| 16 | 6 | Sept. 1 | 2 |

EGG-LAYING HABITS

Egg laying was first witnessed at Willow Creek in 1920. Several gravid females were placed in outdoor observation cages in which a variety of soil conditions and vegetation was offered and were closely watched for several days. On the afternoon of September 4, at 4.45, one of these females was seen laying eggs. She crawled over clods, stubble, and plants, constantly feeling with the ovipositor the objects which she walked upon. On reaching a patch of soft earth she stopped and carefully worked the abdomen into the soil until the wings were flat on the ground. After remaining quiet for a short time she moved and repeated her actions in another spot. Three ovipositions were made in 15 minutes, and after each one the dirt was stirred as the abdomen was withdrawn and the hole left covered with dirt. The dirt around these holes was carefully removed with a teaspoon and eggs were found in clusters of 3 or 4 about ¼ inch below the surface of the ground. A total of 11 eggs were recovered from the three ovipositions.

*Porosagrotis orthogonia* moths were seen laying eggs in the open at Willow Creek on September 5, 1920. Just before dark moths were seen flying over a freshly worked, summer-fallowed field, being most abundant on the higher knolls and along the ridges where the soil was soft and loose. One moth was followed for some distance. She would fly a few feet, never getting over 10 inches above the ground, and would then crawl a short distance, continuously feeling the surface with her ovipositor. On reaching soft dirt she stopped and laid eggs for six minutes, going through the same actions as the moth observed in the cage. When she left the ground she flew straight away for at least ¼ mile at a height of 20 to 30 feet above the ground aud was finally lost to view. Five eggs were recovered from this oviposition. Other moths were seen flying to the ridges and knolls, but it was too dark for further observations on this date. A few days later another moth was observed laying eggs on a knoll in the same field. One oviposition was made which lasted 23 minutes, during which time the moth was not in the least disturbed by any movements of the observer. When the ovipositor was finally withdrawn the moth swung around ¼ inch and started in again, this time remaining quiet for 17 minutes, after which she crawled under a clod to hide. This moth had oviposited for 40 minutes, and 12 eggs were recovered from the two holes. Moths were seen ovipositing along the knolls and ridges in this field for several days.

Moths in egg-laying show their preference for spots in the field where the soil is softest and also indicate a preference for freshly worked fields over those which have become caked and hard. Across the road from the freshly worked, summer-fallowed fields in which egg laying was observed was another summer-fallowed field which was spotted with Russian thistles and in which the soil was caked on the surface, due to a rain some two weeks earlier. Moths were continually observed flying into this field, but they usually flew on across it to the knolls in the freshly worked field, even though it was ¼ mile farther. Very few moths flew to similar knolls in the caked field, and those that alighted hid under the thistles or clods of dirt and made no attempt to lay eggs. Further evidence of this preference for mellow fields will be brought out later in this paper.

## ATTRACTION OF THE MOTHS TO LIGHTS

Our first experiments in attracting the moths to lights were conducted at Wilsall on the evening of August 26, 1919. A large Coleman gas lamp was placed on the ground in the field where the grain had been destroyed the previous spring. As soon as it grew dark *Porosagrotis orthogonia* moths began to come to the light at the rate of one every two or three minutes. The lamp was placed upon bare sandy soil and the ground was well lighted for several feet on all sides. The moths usually struck the

ground from 2 to 15 feet from the light and then crawled toward it, where they could be easily picked up. As soon as it became totally dark the moths came to the light so rapidly that two men could not keep them picked up, and from 9 o'clock until midnight 282 females and 164 males of *P. orthogonia* were placed in rearing cans or killing bottles. This by no means represented the total number that came to the light, for hundreds escaped. Many different species of noctuids were attracted to the light, but fully 95 per cent were *P. orthogonia*. Ten of the females thus captured averaged 132 eggs. (Table II.)

In another part of the same field a Duro moth trap was run throughout the nights of August 26 and 27, 1919, and each morning the pan was well filled with *Porosagrotis orthogonia* moths. During the two nights 4,900 moths were caught, of which 4,200 were males. It is difficult to understand the preponderance of females caught at the larger light between 9 and 12 p. m. and the very small percentage of females caught at the smaller light during the entire night.

Experiments with trap lights were conducted on a somewhat larger scale at Willow Creek. A trap was designed which was made up of utensils commonly found on every farm and which would serve other purposes when not in use as a trap light (Pl. 30, B). It consists of a No. 2 galvanized-iron washtub and a No. 2 barn lantern. In addition, a galvanized-iron arch is made which fits across the tub and serves the dual purpose of deflecting the moths and holding the lantern. When the arch is wired firmly and the lantern swung in place the flame of the lantern is just above the edge of the tub. When set in place, the tub is staked down to prevent its being blown over, and about 4 inches of water are poured into it. About 1/8 to 1/4 inch of kerosene is floated on the water to kill the moths which fall into it.

Eleven such traps were put out at Willow Creek, and observations were made during the flight period of *Porosagrotis orthogonia*. Two traps for catching moths alive were also used, and when these showed that *P. orthogonia* was beginning to fly the tub traps were put out on fields that had been heavily infested with worms.

During the first few nights the catches were small and the moths were counted. The numbers increased nightly until the height of flight, which was from August 19 to 24, inclusive. The night flight gradually decreased after the latter date. When the numbers became too large to count they were estimated, and during the height of flight they were measured in pints. As a pint measure holds from 962 to 1,000 moths, the measuring of moths gave a fairly accurate count. During the heavy flight several of the traps ran as high as 4,000 moths in a single night. The entire season's catch of *Porosagrotis orthogonia* moths in the 11 traps was 82,488. The catch on individual nights is shown in Table V.

TABLE V.—*Number of Porosagrotis orthogonia moths caught at trap lights at Willow Creek, Mont., during the season of 1920*

| Date. | | Weather conditions and remarks | Number of moths caught |
|---|---|---|---|
| Aug. | 12 | Rain in afternoon, night warm, cooler toward midnight | 127 |
| | 13 | Early part of night warmer than usual; warm after midnight | 342 |
| | 14 | Warm all night | 1,528 |
| | 15 | Night moderately warm | 1,566 |
| | 16 | Cooler, especially so after 9.30 p. m.; not as many moths flying | 449 |
| | 17 | High wind in afternoon blew over traps; cold and windy after dark; no moths out | ....... |
| | 18 | Windy; cold soon after dark, almost frost; moths flew only a few minutes | 68 |
| | 19 | Warm at 8 p. m.; moths flying heavily; windy and cooler after 11.30; fewer moths out | 11,720 |
| | 20 | Warm west wind most of night; heavy flight of moths | 13,990 |
| | 21 | Same as night before, height of flight 9.15 p. m. | 14,950 |
| | 22 | Warm, light west wind | 13,650 |
| | 23 | Warm, west wind, wind strong and cold after 12.30 a. m. | 16,250 |
| | 24 | Cold and cloudy after 9 p. m.; southwest wind | 7,490 |
| | 25 | Windy and cold; moonlight, no moths flying | ..... |
| | 26 | Windy until 8 p. m., clear, cold, full moon; no moths flying | ..... |
| | 27 | Wind and heavy rain; cold; no moths | ..... |
| | 28 | Rain all morning; cold, windy night, traps not lighted | ..... |
| | 29 | Cool; little wind, few moths flying early, too cold after 9 p. m. | 68 |
| | 30 | Clear, cool; bright moon; no moths flying | ..... |
| | 31 | Cloudy to 9.30; clear, cold; bright moon | 110 |
| Sept. | 1 | Moths flying in daylight afternoon, few flying after dark | 171 |
| | 2 | No moths flying after dark; a few found feeding | ..... |
| | 3 | Few moths out after dark, not attracted to lights | ..... |
| | 4 | Three *Noctua c-nigrum* caught; no others flying to light | ..... |
| | 5 | No moths flying to traps; traps taken up | ..... |
| | | Total | 82,488 |

In all observations made at Willow Creek no moth was ever seen to land on the ground on its way to a light trap as did the moths at Wilsall the previous year. This may have been due to the fact that the tub hid the light so it would not strike the ground, and in order to keep in the path of light the moth had to fly straight to the trap. This was usually the case, and for the most part moths flying to the traps came on a straight line from 4 to 15 feet above the ground. They either struck the arch or lantern or went straight on over the trap.

On a still, dark, fairly warm night the moths would come to the traps in varying waves of abundance for which there was no apparent reason. There would be a cloud of moths for a few minutes and then they would come in scattering two's or three's. If the wind was strong no moths were caught in the traps and no moths could be found moving about on the ground. No moths were caught during a rain or ever after a rain, as long as the ground and vegetation remained wet. When the moon was bright, moths were not caught nor were any seen flying, though they would start the minute the moon went behind a cloud. Practically no moths were caught after the temperature had dropped below 58° F.

### MOTHS BOTH NOCTURNAL AND DIURNAL

During the last half of August when the nights were warm and night ying was at its height the moths remained inactive during the day, iding under clods and weeds. As the nights grew colder, the moths ew only an hour or two after dark, and on September 1 they were seen ying during the day. On this date they began flying about 4.30 p. m. nd were seen in abundance feeding upon sunflowers, golden rod, umbling mustard, yellow greasewood and lamb's quarter. All but one f the moths seen at flowers at this time were males, a search under eeds and clods at the same time revealing only females. At 8 p. m. hen the flower patches were visited moths were still feeding in large umbers, practically all of them being females. They paid no attention lights, and none were caught in a trap light set close by. As the night rew colder all of the moths disappeared and could be found hiding under ods or weeds. On the following day moths were found feeding at owers at 1 p. m., and at 3.30 p. m. a patch of yellow greasewood *hrysanthus frigidus*), which seemed to be the favorite flower, had ttracted dozens of *Porosagrotis orthogonia*, nearly all of which were ales. At 5.30 p. m. the patch was again visited, and it was found that e males were then leaving and that females were flying to the flowers om a nearby summer-fallowed field. On September 3 moths of various pecies were found feeding during the morning, and at noon the flowers the yellow greasewood were covered with moths, none of which were *. orthogonia*. At 3 p. m. about 10 per cent of the moths present at owers were *P. orthogonia*. These gradually increased in numbers until 15 p. m., at which time practically all other species had disappeared. fter the moths had finished feeding they invariably flew toward the igher ridges and knolls in neighboring cultivated fields. Many of the oths in coming to the flowers were seen to fly from 200 to 500 yards irectly against a stiff breeze. Moths were seen flying to flowers in large umbers until September 8, when a cold rain and wind occurred. The ales were always found feeding earlier in the day and the females later, lthough neither was ever found before noon.

### ECONOMIC IMPORTANCE

The record of this cutworm during the last 10 years has demonstrated s capacity for doing enormous damage to grain crops. When in 1911 *orosagrotis orthogonia*, then an obscure insect, suddenly increased in umbers and did considerable damage to grain in southern Alberta (*10, p.* 77) little importance was attached to it. In the following year, however, hen 33 per cent of all the grain sown in the Lethbridge land district as destroyed and an accurate estimate by the superintendent of the xperiment station at Lethbridge placed the actual loss from this insect from 30,000 to 35,000 acres (*11, p. 506*) it was looked upon as a pest of

major importance. During the last two years *P. orthogonia* has been r sponsible for losses in central Canada amounting to several million dollar

In Montana the pale western cutworm has been on the increase sin it was first noticed in 1915 and is now the most destructive insect pe with which the grain grower has to contend. In 1915 at Conrad 80 acr of wheat were destroyed and were reseeded to oats, which was also take Flax was then seeded, but this also was so badly injured that the own plowed the field and summer fallowed it. This instance was typical scores of losses in the district now composed of the counties of Choutea Teton, and Pondera.

During the next two years, especially in 1917, great losses were su tained throughout the north central portion of the State, due to cu worms which worked entirely beneath the surface of the soil and whi were doubtless no other than *Porosagrotis orthogonia*. In 1919 the pa western cutworm appeared in destructive numbers farther south an caused severe losses in Park and Jefferson Counties as well as in th previously infested area. A conservative estimate of the losses for th year based on the reports of county agents, hundreds of questionnair returned by farmers, and the personal investigation of many fields different parts of the State, is at least 200,000 acres. In 1920 the injur in Jefferson and Park Counties was more widely extended, and there w a decided increase in the damage done in many of the districts previous infested. The loss over the entire State for the year is placed at 250,00 acres, valued at $3,000,000. In the Willow Creek district in Jeffers County a careful survey conducted in 1920 showed that 29 per cent the total seeded area had been destroyed by this cutworm, and a simil survey in several of the northern counties showed a loss of 35 per cent the grain crops planted.

To show perhaps a little more clearly what this cutworm has bee doing it may be stated that 100 fields personally inspected during th summer showed a loss of 2,437 acres out of a total of 6,844 in 1919, an in 1920 a loss of 3,382 acres out of 6,844, or 35.7 per cent in 1919 an 49.4 per cent in 1920. Mr. George O. Sanford, manager of the Sun Riv irrigation project, has stated to us that of the 15,300 acres seeded to cro on the Greenfield Bench in 1920, 7,345 acres was a total loss and tha some damage was done to the remainder. Using the figures he has give for the average yields on the undestroyed acreage—wheat 11.5 bushel oats 20.86 bushels, and flax 6.31 bushels—the average value of the pri cipal farm crops of that section was at least $15 per acre. Accordingl using that as a fair valuation per acre of the crops destroyed, the pa western cutworm inflicted a loss of $110,175 in this one comparative small territory. Although these losses took place on irrigable land, n water was available until after the first of June. Were it not that irr gation made it possible in some cases to reseed and grow a late crop o part of the originally destroyed area, the loss would have been 55 pe cent instead of 48 per cent of the acreage in that district.

## EXPERIMENTS IN CONTROL

### ORDINARY CUTWORM CONTROL METHODS NOT EFFECTIVE

Early in our study of *Porosagrotis orthogonia* it became apparent that the ordinary method of scattering poisoned bran mash over an infested field was not effective in controlling this species. On May 2, 1919, poisoned bran mash was scattered over a heavily infested field in southern Montana at the rate of 20 pounds to the acre and was followed by three other applications on successive days. The field was examined each day by the owner, who reported that he could not find a single dead worm. On May 7 the treated area was carefully examined by one of the writers but no dead worms could be found, nor could any decrease in the number of live worms be noted. On May 7, 1919, poisoned bran mash was scattered over 2 acres of heavily infested wheat in northern Montana. During the next 10 days no results whatever were secured from this treatment. County agents and numerous farmers have reported that attempts to poison this species by the ordinary method of scattering poisoned bran mash over the surface have always resulted in failure.

At Willow Creek in 1920 pale western cutworms were noticed crawling over the surface of the ground in the evening after a rain, and an attempt was made to kill them by scattering poisoned bran mash during the night. The bait was scattered soon after dark over an area which included bare ground, scattering wheat, and a good stand of wheat, all heavily infested. Observations were made during the night by the aid of automobile headlights, and many of the worms were seen feeding upon the bait. Two days later a search was made for dead cutworms. In the area where there was no vegetation it was estimated that 60 per cent of the worms were killed; where there was a scattering of wheat the percentage of dead worms was 50; and where there was a good stand of wheat 43 per cent were killed. It is possible that several night applications of poisoned bran mash during rainy weather might bring about a satisfactory control, but as yet we have not had the opportunity to try it.

### POISONED BRAN MASH HARROWED INTO THE SOIL

Strickland reports (17) that poisoned bran mash harrowed into the soil gave gratifying results. This method was tried out at Wilsall in May, 1919. Poisoned bran mash was scattered over ½ acre of heavily infested wheat at the rate of 25 pounds to the acre. On several square rods where the worms were thickest the mash was worked well into the soil with a hand rake, and the remainder of the treated area was thoroughly worked with a spike-toothed harrow. The plot was carefully examined three days after the poisoned bran mash was applied, and it was estimated that the treatment was not more than 1 per cent effective. Very few dead cutworms could be found, and eventually all of the wheat was destroyed.

### POISONED BRAN MASH DRILLED INTO THE SOIL

Since *Porosagrotis orthogonia* very rarely comes to the surface to feed, placing the poisoned bran mash beneath the soil was tried in the hope that the cutworms would thus come in contact with it and feed upon it. The most promising way of doing this seemed to be with a seed drill. This method was tried out in northern Montana at Havre and in southern Montana at Wilsall.

#### TESTS AT HAVRE

At Havre two formulæ were used.

FORMULA NO. 1

| | |
|---|---|
| Shorts ....pounds.. | 25 |
| Paris green ..do... | 1 |
| Oranges | 4 |
| Molasses .quarts . | 2 |
| Water ....gallon.. | 1 |

FORMULA NO. 2

| | |
|---|---|
| Shorts....pounds.. | 25 |
| Paris green .do.... | 1 |
| Molasses ..gallon.. | 1 |
| Water .quarts.. | 2 |

These mixtures after being prepared were spread out and allowed to dry for 24 hours. When dry, No. 2 was distinctly stronger smelling, although both had a good molasses odor. The reason for using the large amount of molasses in these formulae was to secure a distinct odor in the dried material which we hoped might attract cutworms in the soil for some little distance.

The dried material was seeded into the ground with a Van Brunt drill at the rate of 16 pounds to the acre and at a depth of about 1.5 inches. Six acres were treated. The drill was run at right angles to the rows of grain so that the worms in working from plant to plant would only move a few inches before coming in contact with the bran. The greatest difficulty encountered was in getting the bran to feed evenly through the drill. When it was sufficiently dry to be well divided it was too light to force its way through and it was necessary to agitate the mixture continuously in the seeder box to get anywhere near an even distribution.

The field was examined two days after the poisoned bran mash was drilled in, and it was found that formula No. 1 had killed approximately 50 per cent of the worms while formula No. 2 gave slightly better results with a kill of about 55 per cent, which was not enough to prevent the destruction of the crop.

TESTS AT WILSALL

On June 8, 1919, a similar test was conducted at Wilsall. The following formula was used:

| | |
|---|---|
| Shorts | pounds.. 25 |
| Paris green | do.... ½ |
| Salt | do.... ½ |
| Molasses | quarts. 2 |
| Water | gallon 1 |

After mixing, the mash was spread out to dry, which with a hot sun and a fair breeze was accomplished in half a day. The mixture was distributed over 25 acres of infested wheat at the rate of 12 pounds of the dry mash to the acre. Sixteen acres were sown with all the spouts of the drill working and 9 acres with every other one closed. The drill was run across the old grain rows. The greatest difficulty encountered was the same as in the test at Havre—the mixture was too light to feed evenly through the drill. This was overcome by using two men on the drill, one to drive and one to keep the bran shaken down where it would come in contact with the disks of the drill. This was done by frequently pounding the seeder box with a padded hammer and punching out packed masses with a small stick. Dead and dying worms were found the second day after the poisoned bran was drilled in, and on the third day a careful examination was made and it was estimated that from 50 to 60 per cent of the worms had been killed. The field was examined two weeks later, and there was a very noticeable difference in the number of worms found in the treated and untreated areas, but this did not prevent total destruction of the crop.

TEST AT WILLOW CREEK

During May, 1920, poisoned bran mash was distributed with a grain drill over a very badly infested field at Willow Creek. Cutworms were uniformly scattered over a 40-acre field of spring wheat, and at the time the poisoned bran mash was applied had destroyed about half the plants. Conditions were ideal for a good test of control methods. The following mixtures were used:

FORMULA NO. 1

| | |
|---|---|
| Shorts | pounds.. 25 |
| Paris green | do... 1 |
| Molasses | quarts.. 2 |
| Salt | pound. 1 |
| Water | gallon.. 1 |

After mixing, this was thoroughly dried ont and was then seeded 2 inches deep through a Van Brunt drill at the rate of 20 pounds to the acre. Two acres were sown. This mixture did not feed uniformly through the drill unless constantly agitated.

FORMULA NO. 2

| | | |
|---|---|---|
| Shorts | pounds | 25 |
| White arsenic | do | 1½ |
| Molasses | quarts | 2 |
| Salt | pound | 1 |

This was prepared and distributed in the same manner as formula No. 1. Two acres were sown.

FORMULA NO. 3

| | | |
|---|---|---|
| Shorts | pounds | 25 |
| Paris green | do | 1 |
| Salt | do | 1 |

This was mixed dry and seeded at the rate of 12½ pounds to the acre. It ran through the drill about the same as the mixtures which were mixed wet and then dried. Two acres were sown.

FORMULA NO. 4

| | | |
|---|---|---|
| Shorts | pounds | 25 |
| White arsenic | do | 1½ |
| Salt | do | 1 |

This was prepared dry and then thoroughly mixed with an equal bulk of wheat. This combination ran through the drill very evenly, the wheat being heavy enough to carry the bran through the drill without clogging. Two acres were seeded at the rate of 12½ pounds of bran to the acre. Three days after the poisoned bran was put out the field was examined and it was estimated that about 10 per cent of the worms in the treated areas had been killed. No difference could be seen in the effectiveness of the various formulæ, and numerous living cutworms remained in all the plots. One week later the plots were again examined and the number of dead cutworms had not materially increased. A final examination of the field was made on June 14, three weeks after the poisoned bran was put out. Cutworms were found in abundance on all plots, and in plots 1, 2, and 3 practically every spear of wheat had disappeared. In plot 4, which was seeded with a mixture of wheat and poisoned bran, the wheat was about 3 inches in height and was being rapidly cut off, 50 per cent of the new stand being already destroyed. From a practical standpoint the control on all plots was a complete failure and an absolute waste of materials.

## POISONED BAIT SPRAY FOR ADULTS

The presence of large numbers of *Porosagrotis orthogonia* moths at flowers led us to try out the following poisoned bait spray:

| | | |
|---|---|---|
| Water | gallon | 1 |
| Molasses | pint | ½ |
| White arsenic | ounce | ⅛ |
| Amyl acetate | do | ⅕ |

This was scattered in coarse droplets over flowers and vegetation where moths were abundant. Many flies and bees were killed, but no moths were observed feeding upon the bait, and dead moths were never found in the vicinity of the sprayed vegetation.

## CULTURAL METHODS AS A MEANS OF CONTROL

In our study of *Porosagrotis orthogonia* under field conditions we have repeatedly noticed instances where crops in one field were completely destroyed, while in an adjacent field the grain escaped unharmed. This suggested that the manner in which the ground was worked before the crop was put in might have been responsible for the great difference in the amount of damage done in the two fields, and in 1920 a survey was conducted with the object of determining the relation of cultural methods to cutworm abundance. This survey was conducted in two ways: (1) By an auto trip through the districts most heavily infested by means of which hundreds of farmers were personally interviewed and the histories of their fields obtained for the period 1919–20; (2) by questionnaires sent to all farm bureau members in counties where *Porosagrotis orthogonia* was known to be present.

The percentage of cutworm losses under various cultural methods as shown by a study of fields, the owners of which were personally interviewed, is shown in Table VI.

TABLE VI.—*Percentage of Porosagrotis orthogonia injury in 1920 under various methods of cultivation in preparation for seeding*

| Cultivation between previous crop and 1920 crop | Number of fields. | Total acres. | Acres lost. | Percentage lost. |
|---|---|---|---|---|
| Fall double disked | 8 | 465 | 200 | 43.0 |
| Spring double disked | 39 | 2,250 | 1,301 | 57.0 |
| Spring single disked | 36 | 1,536 | 661 | 43.0 |
| Fall-plowed; disked or harrowed before seeding | 13 | 643 | 138 | 21.0 |
| Spring-plowed; disked or harrowed before seeding | 51 | 2,465 | 666 | 27.0 |
| Spring-harrowed | 18 | 1,332 | 526 | 40.0 |
| Summer-fallowed | 39 | 3,114 | 267 | 8.5 |

A study of the results shows a high percentage of cutworm injury in all cases where the stubble was only disked or harrowed before seeding. Fields which were plowed either in the fall or spring showed a somewhat lower percentage, while summer-fallowed fields showed only the very small loss of 8.5 per cent.

While the average cutworm loss in summer-fallowed fields was low, yet several individual fields suffered severe losses. It was therefore decided to make a study of the histories of summer-fallowed fields during the two seasons of 1919 and 1920. Since the moths were known to

prefer loose mellow soil for egg-laying, it was thought that the condition of the ground in summer-fallowed fields during the egg-laying period might have considerable influence on the number of eggs deposited in the field and on the percentage of loss the following spring. Since egg laying does not begin until about August 15, fields which are not cultivated or disturbed in any way after July 15 become more or less crusted and caked. Fields which are cultivated in any way during the last part of July or during August, on the other hand, are very likely to be soft and mellow during the egg-laying period, thus offering the very conditions which the moths are seeking. Forty-eight fields, for which we had data for both 1919 and 1920, were therefore classified as crusted, if they were worked only before July 15, or as mellow, if they had been worked after that date. The percentage of loss for the variously worked fields is shown in Table VII.

TABLE VII.—*Percentage of Porosagrotis orthogonia injury during 1919 and 1920 in "crusted" and "mellow" summer-fallowed fields. Data secured by personal interview with grower*

| Condition of field and time of cultivation. | Number of fields. | Total acres. | Number of fields infested. | Acres lost. | Percentage lost. |
|---|---|---|---|---|---|
| "Crusted"—worked only before July 15 | 27 | 1,828 | 3 | 14 | 00.7 |
| "Mellow"—worked after July 15 | 21 | 1,562 | 14 | 425 | 27.2 |

Farmers were asked in questionnaires sent to farm bureau members in counties infested with *Porosagrotis orthogonia* whether they had noticed any relation between the condition of the soil in summer-fallowed fields during August and the amount of pale western cutworm injury the following spring. Sixty-eight grain growers answered this question. Fifty-three said that injury was most severe in fields where the surface soil was well pulverized, or, as one farmer stated it, "The more mulch the more worms." Seven reported that the greatest injury had occurred in fields that had been crusted during August, and five stated that they could see no relation between soil conditions and cutworm injury.

The foregoing data, together with the fact that we have seen ovipositing females show a distinct preference for mellow fields, leads us to the conclusion that the physical condition of the soil during the egg-laying period has a very important bearing upon the amount of *Porosagrotis orthogonia* injury that may occur the following spring. According to the data at hand greatest injury may be expected in fields in which the surface soil is loose and well pulverized during the egg-laying period. This loose, mellow condition may have been brought about in summer-fallowed fields by tillage during late July and August or it may

be a natural condition such as is found on knolls and ridges where the soil is generally light and easily drifted. In fields where a crop is removed during July or August the surface crust may become broken and pulverized in numerous places by the disturbance of the soil in connection with harvesting, thus offering the moths many desirable spots for egg laying. Injury may be least expected to occur in fields in which the surface soil is hard or crusted during the egg-laying period. In most instances this condition can be brought about by not disturbing the ground in any way between July 15 and September 15. If farmers in preparing their grain fields for seeding will be governed by these principles it is believed that *Porosagrotis orthogonia* injury can be greatly reduced. Fortunately this method of handling summer-fallowed fields does not interfere with approved farm practices, and in fact agrees very closely with the recommendations of the agronomists.

## NATURAL ENEMIES

Unlike most of our common cutworms, *Porosagrotis orthogonia* suffers comparatively little from attack by natural enemies. Much difficulty has been encountered in rearing various other species taken in the field as larvæ, particularly the army cutworm, *Chorizagrotis auxiliaris*, on account of the high percentage that developed disease or parasites. This has not been the case with the present species. Our records for 1915 show that out of a large number of army cutworms reared individually only 35 per cent were brought through to the moth stage, parasites emerged from 24 per cent, 21 per cent died of disease, and the remaining 20 per cent died in the pupa stage, mostly on account of insect parasites. In 1919, 55 per cent of *P. orthogonia* larvæ handled in the same way were reared to adults. Of the 45 per cent that died, very few seemed to die of any disease, and parasites emerged from only two larvæ.

In 1920, out of 960 *Porosagrotis orthogonia* larvæ collected in the field, 13.7 per cent were parasitized, 12.2 per cent by Diptera and 1.5 per cent by Hymenoptera. The parasites which emerged were 14 *Bonnetia compta* Fall and 1 *Peleteria robusta* Wied.

The common wild birds of the prairie are the most beneficial natural check that we have observed. The western grasshopper sparrow, *Ammodramus savannarum bimaculatus* Swainson, particularly, has been watched while digging out the larvæ and carrying them away to its young. In many parts of the cutworm-infested regions it has been a common sight toward the last of June to see thousands of small excavations made by the western grasshopper sparrow, horned larks *Otocoris alpestris leucolaema* Coues (Pl. 30, C), and possibly other wild birds in their search for the larvæ.

Although the common ground squirrel, *Citellus richardsoni* Sabine, has at times been known to seek out and devour large numbers of cutworm

larvæ, we do not believe that ground squirrels are of much importance as a natural check.

In some instances both larvae and adults of *Calosoma tepidum* Lec. have been observed to be especially numerous about cutworm-infested fields and are, we believe, one of the lesser important predators.

## DESCRIPTION OF STAGES

### EGG

Spheroidal, flattened dorso-ventrally, glistening milk-white when first laid (Pl. C, 1), later becoming dull gray; 1 mm. in diameter, .8 mm. in height. Around the micropyle is the usual rosette which lies in the center of a finely reticulated area about 0.3 mm. in diameter. The pattern of the reticulation is shown in figure 1. From the edge of the reticulated area about 30 slightly raised longitudinal ribs radiate toward the base, extending approximately four-fifths the distance from the apex to the base. The ribs are sometimes irregularly branched or connected by cross ridges. The shallow channels between the ribs are transversely striated and lightly pitted. The chorion on the basal fifth of the egg is smooth and glistening without ridges or definite reticulation.

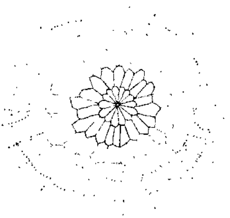

FIG. 1.—*Porosagrotis orthogonia*, reticulation about the micropyle. Highly magnified.

### LARVA [1]

#### FIRST INSTAR

Head width, 0.4 to 0.43 mm.; average 0.41 mm.

Total length of body, 2.4 to 3 mm.; average 2.8 mm.

The head (Pl. 28, A) is a very dark glistening brown, almost black; clypeus and front same color as epicranium; adfrontals indistinct, but apparently extending almost to deep indentations at summit; mandibles, labrum, ring-shaped sclerite resting on mentum, and cardo dark brown; submentum, margins of stipes next the mentum, the antennae, and labial palpae brownish; ocellar region blackish.

The posterior portion of the thoracic shield dark brown; anterior margin very light brown. The thoracic legs are brownish with dark brown transverse lines immediately anterior and partially surrounding coxæ.

[1] In the description of the larva the naming of the various parts follows very largely the system of William T. M. Forbes (4).

Prolegs on segments 9, 10, and 13 (counting the head as the first segment); crotchets brown; rudimentary prolegs slightly visible as small tubercles on segment 8.

There is a reddish brown mottling over the lateral and dorsal regions, becoming more distinct along the posterior segments. Before the larva has taken food this coloration appears much darker. The general color of the newly hatched larva is brown. The spiracles dark brown, setæ *single and arising from minute brownish tubercles.*

SECOND INSTAR

Head width, 0.47 to 0.576 mm.; average 0.53 mm.

Total length of body, 2.8 to 3.77 mm.; average 3.3 mm.

The head (Pl. 28, B) remains a dark, shiny brown; mandibles and labrum dark brown, mandibles at teeth and labrum at notch blackish; ring-shaped sclerite resting on mentum blackened ventrally and with its setæ within the sclerite; cardo dark brown; submentum, margins of stipes next the mentum, antennæ, and labial palpæ brownish; ocellar region blackish.

Thoracic shield dark brown posteriorly, but not as dark as head; anterior margin light brown. On each side of the light brown dorsal stripe is a dark spot on the anterior portion of the shield with several dark spots laterad.

Prolegs on segments 8, 9, 10, and 13; crotchets on eighth segment consisting of only two or three hooks; rudimentary prolegs on segment 7 beginning to show; crotchets reddish brown.

The dorsal stripe is gray, bordered with broken lines of brown; subdorsal and lateral stripes brownish; spiracles dark brown; setæ single, tubercles brownish.

THIRD INSTAR

Head width, 0.68 to 0.786 mm.; average 0.75 mm.

Total length of body, 4.19 to 5.5 mm.; average 4.29 mm.

Head brown (Pl. 28, C) with upper parts of lobes of epicranium dark brown; front brown but with its lower margin together with clypeus dark brown; mandibles reddish brown to black at teeth; labrum dark brown with blackened notch, ring-shaped sclerite resting on mentum blackened ventrally and with its setæ within sclerite; cardo dark brown; submentum, margins of stipes next the mentum, antennæ and labial palpæ brownish; ocellar region blackish.

Thoracic shield dark brown, almost black at posterior region; anteriorly it is a lighter brownish gray with a small black spot on either side of the light dorsal stripe and with several dark spots laterad.

Thoracic legs tinged with brown, claws and markings anterior to and partially surrounding basal joints reddish brown.

The dorsal stripe along the body is made of broken gray which is bordered irregularly with brown. Subdorsal and lateral stripes brownish; general color same as dorsal stripe, or lighter, with a greenish tinge. Spiracles dark brown; setæ single, tubercles greenish brown.

FOURTH INSTAR

Head width, 0.84 to 1.14 mm.; average 1.02 mm.

Total length of body, 6.5 to 12.5 mm.; average 8.9 mm.

Coloration of head slightly modified from preceding instar; front not as dark, clypeus a lighter brown, and cranial lobes considerably darker at top (Pl. 28, D); mandibles are black at teeth and fade to reddish brown to dark brown near articulations; labrum dark brown with blackened notch; ring-shaped sclerite resting on mentum blackened ventrally and with its setæ within the sclerite; cardo dark brown; submentum, margins of stipes next the mentum, antennæ and labial palpæ brownish; ocellar region blackish.

Posterior part of thoracic shield dark brown; anteriorly it is lighter, and in this lighter area are three distinct dark spots on either side of the dorsal line and also larger dark spots toward the lateral ends of the shield.

Thoracic legs tinged with brown, claws and markings anterior to and partially surrounding basal joints, reddish brown.

Prolegs on segments 8, 9, 10, and 13 and rudimentary prolegs on segment 7; crotchets reddish brown.

The dorsal line is a greenish gray partially broken and bordered with brown; subdorsal and lateral lines brownish; general color green to gray; spiracles dark brown; tubercles are greenish, setæ single and ringed at base with a light color.

FIFTH INSTAR

Head width, 1.38 mm. to 1.98 mm.; average 1.83 mm.

Total length of body, 11.5 mm. to 18.0 mm.; average 16.1 mm.

General color of head much lighter (Pl. 28, E); ocellar region very dark brown; ocelli 1, 2, and 6 colorless, other three dark; mandibles reddish brown to black at teeth; lower margin of labrum reddish, blackened at notch; cardo and submentum brown with the margins of stipes next the mentum same color and with the stripe becoming wider about the base of the palpifer; sclerite resting on mentum blackened.

The two bands of dark brown on the epicranium and bordering the adfrontals become prominent for the first time in this instar (Pl. C, 2). The rest of the head seems to have lost color, leaving these two stripes which run from points even with the base of the clypeus to the second epicranial setæ, above which they gradually fade out about the first epicranial setæ.

'he thoracic shield is brownish with a light dorsal stripe; in the lighter ı on the anterior margin of the shield and on either side of the dorsal pe are distinct blackened spots, with other dark spots toward the ral margins of the shield.

'horacic legs tinged with brown, claws and markings anterior to and tially surrounding basal joints reddish brown.

ully developed prolegs on segments 7, 8, 9, 10, and 13 are concolorous ı body; crotchets brownish. The anal plate is marked with a trans-se row of small brownish spots anterior to the setæ.

'he dorsal line is a greenish gray and bordered with brown; the sub-sal and lateral stripes brownish; the general color is about the same perhaps a trifle lighter than the dorsal stripe.

SIXTH INSTAR

Iead width, 1.98 to 2.64 mm.; average 2.41 mm.

'otal length of body, 2.0 to 2.5 cm.; average 2.2 cm.

Iead light brown; ocellar region very dark brown; ocelli 1, 2, and 6 ısparent, other three brownish; mandibles reddish brown to black eeth; lower margin of labrum reddish, blackened at notch; cardo and mentum brown with the margins of stipes next the mentum same r with the stripe broadening apically about the base of the palpifer. 'he two conspicuous bands of dark brown persist on the epicranium dering the adfrontals (Pl. 28, F); the stripes become a lighter color op, ending near the first epicranial setæ; a denser colored portion of h band follows the adfrontals almost to their apex.

'he thoracic shield is brownish with a light dorsal stripe; in the lighter ı on the anterior margin of the shield and on either side of the dorsal pe are distinct blackened spots with other dark spots toward the ral margins of the shield.

'he thoracic legs tinged with hrown, claws and markings anterior to partially surrounding basal joints reddish brown.

'ully developed prolegs are found on segments 7, 8, 9, 10, and 13, con-rous with body; crotchets brownish. The anal plate on the thirteenth ment, a pale green in color, possesses a transverse row of brownish ts anterior to the setæ; a light-colored dorsal stripe runs through the te.

'he dorsal stripe is a broken gray green bordered with light brown; subdorsal is brownish, but the lateral has become lighter in color, wnish gray. Setæ single, ringed at base with a light-colored area; ercles greenish brown; spiracles black.

SEVENTH INSTAR

Iead width, 2.70 to 3.18 mm.; average 2.93 mm.

'otal length of body, 2.9 to 3.2 cm.; average 3.02 cm.

Iead light brown; ocellar region very dark brown, ocelli 1, 2, and 6 nsparent, other three brownish; mandibles reddish brown to black at

teeth; lower margin of labrum reddish; ringed-shaped sclerite resting c mentum blackened; cardo and submentum brown with margins of stipe next the mentum, same color with the stripe broadening about the ba of the palpifer.

The bands of dark brown bordering the adfrontals have the sam appearance as in the previous instar (Pl. 29, A).

The thoracic shield is dark brown with a distinct light-colored dors stripe on either side of which toward the anterior margin of the shiel is a small blackened area with other dark spots toward the lateral margi of the shield.

The thoracic legs are tinged slightly with brown toward the apic joints; claws reddish brown, and color markings remain the same abo the basal joints. Prolegs on segments 7, 8, 9, 10, and 13 are concoloro crotchets brownish; the anal plate is a gray green with dark spots in transverse row anterior to the setæ; a light-colored dorsal stripe ru through the plate.

The dorsal stripe is gray-green, and the brown borders in the previo instars appear greenish in this one; the subdorsal and lateral stripes a greenish with a slight tinge of brown; the general color is a greenish gra setæ single and ringed at base with light area; spiracles black, tubercl green; the pulsating dorsal vessel can be easily seen through the ep dermis.

### EIGHTH INSTAR

Head width, 3.18 to 3.42 mm.; average 3.36 mm.

Total length of body, 3.1 to 3.6 cm.; average 3.34 cm.

The general color of the head is a light brown with a slight yellowis tinge; ocellar region dark brown, ocelli 1, 2, and 6 colorless, other thr dark brown to black; the front is a trifle lighter in color than the clypeu the adfrontals, which are made very distinct by a darker brown coloratio following the frontal sutures and by the dark bands following the ep cranial sutures, extend to the bottom of the deep indentation separati the epicranial lobes at the summit; the mandibles are black at teeth an at points of articulation, and between lies an area which is a very pa brown in color; submentum and cardo brown; chitinized brownish strip on margin of stipes, next the mentum, broadened about base of palpife antennæ, labial and maxillary palpæ brownish.

The two bands of dark brown which border the adfrontals and whic are very conspicuous in the fifth, sixth, and seventh instars, are som what reduced, especially the upper parts of the bands in the regions c the first and second setæ, in the advanced stage of the last instar (P 29, B); during the first days of the instar the bands extend from point even with the base of the clypeus to the second epicranial setæ, runnin between the setæ and the adfrontals; here they become slightly le dense in color and divided, parts of the stripes continuing along th

adfrontals and the other, lighter but wider parts, extending back to the regions of the first epicranial setæ.

The thoracic shield is pale brown with a prominent whitish dorsal stripe; there are small spotted dark brown areas toward the lateral margins of the shield and several smaller brown spots on either side of the dorsal stripe.

The thoracic legs are tinged with brown, especially laterad; about the base of the coxæ and femora anteriorly are reddish brown stripes.

The prolegs on segments 7, 8, 9, 10, and 13 are concolorous with body and possess reddish brown crotchets.

In the first part of this instar (Pl. C, 3) there are distinct dorsal and subdorsal stripes, the dorsal appearing as dark green and produced by the pulsating dorsal vessel beneath the epidermis, and subdorsal as brownish: a broken whitish lateral stripe is quite distinct; in the advanced stage of the instar with the exception of the tubercles there are no markings on the body, which becomes a bleached out yellowish color.

The spiracles are black; setæ, which are a reddish brown especially in the head region, are single; tubercles a greenish brown but immediately about the base of each seta there is a ring of lighter color; the anal plate is marked by a transverse row of dark brown spots anterior to the setæ.

PUPA

Length 17.5 mm., width 5.7 mm.

Typical noctuid pupa; labial palpæ exposed for entire length; maxillæ, mesothoracic legs and antennæ of practically same length and extending almost to caudal margin of wings; prothoracic femora exposed; tips of metathoracic legs visible and mesothoracic legs not extending to eye pieces; dorsal cephalic margins of abdominal segments 5, 6, and 7 marked with many small chitinized circular pits which extend to ventral surfaces of segments but where they are fewer in number and less prominent. The slightly bifurcate, blackish, rough cremaster ends in two stout often incurved spines set far apart. The color of pupa varies from a light straw color to a dark brown, according to age (Pl. C, 4).

ADULT [1]

"*Agrotis orthogonia*" nov. sp.

All the tibiae spinose. Antennae of the male strongly serrate. Middle of the second joint of palpi black, its outer edge and tip, as well as the third joint, light. Head and thorax gray. Anterior wings dark gray; all the markings well expressed; half-line followed by a white shade line; basal space lighter than the other portions of the wing; interior line forming a very long outward projection below the submedian vein, and another shorter one on the costa, the line is white and distinct, bordered with black on each side, between the submedian and subcostal veins it is straight, except one lobe below the median vein, to which the concolorous, black edged claviform spot is attached; subcostal median and submedian veins white, and contrasting

[1] The description of the adult is quoted from Morrison (*15*).

(Pl. C, 5); orbicular spot elliptical, with an outer black ring, within which appears a white annulus, inclosing the gray center; reniform spot large and of the usual shape, the portion of its black annulus, beneath the median vein, separated and very distinct, exterior line rounded, formed of interspaced luniform marks, followed by a white shade line, subterminal space rather lighter than the median space, terminal space again dark, a series of partially effaced cuneiform marks, before the white subterminal line, which forms two short teeth on the second and third median branches. Posterior wings whitish at the base, with a black terminal band and contrasting white fringes. Beneath whitish, the center of the median space dark, and the neighborhood of the median vein, on the anterior wings, clothed with long soft hair.

Expanse, 34 mm.

Hab Glencoe, Nebraska. Received from Mr. G. M. Dodge. (No.66).

The nearest ally of this fine species is the European *Agrotis vestigiales* Rott.

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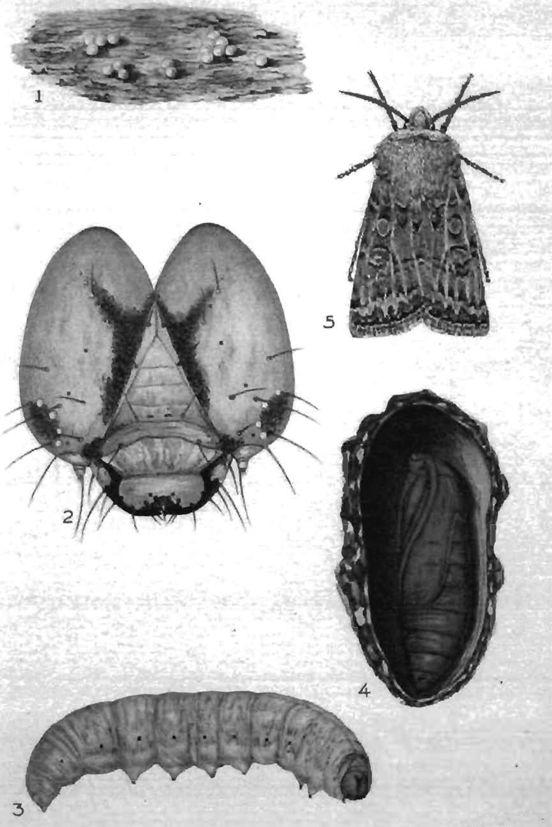
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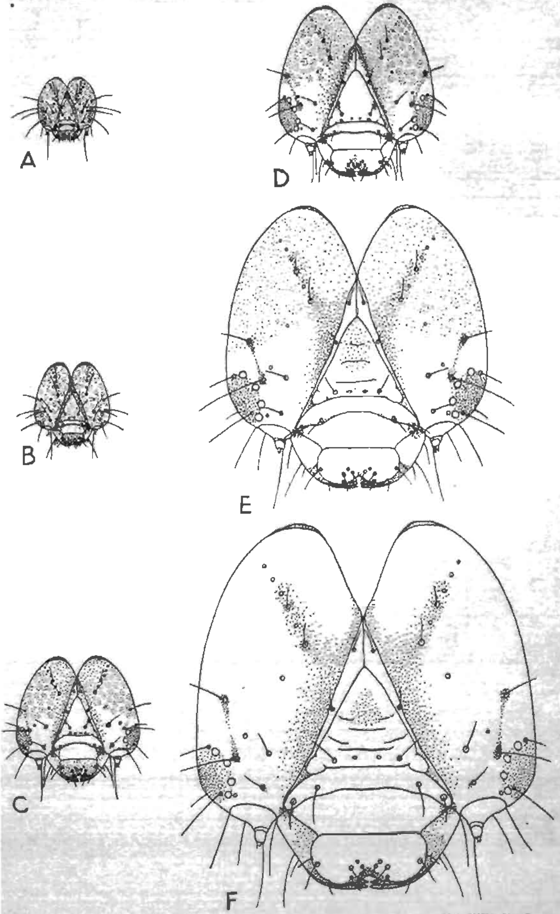
PLATE 28

*Porosagrotis orthogonia:*

A, B, C, D, E, F.—Cast heads of first- to sixth-instar larvæ.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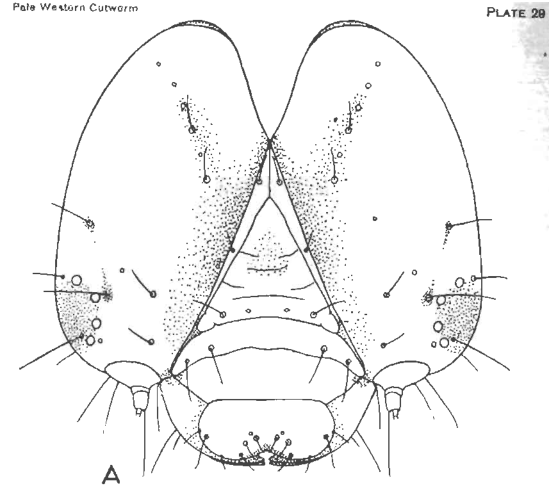

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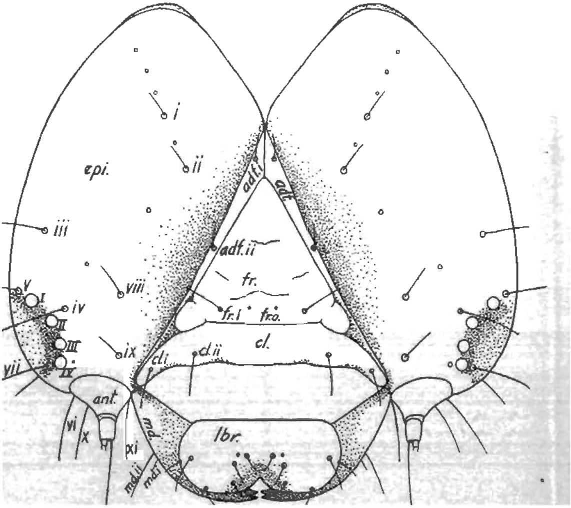
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cl.
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cl.ii
ant.
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x
xi
md.
lbr.
md.ii
md.i

## PLATE 29

*Porosagrotis orthogonia:*

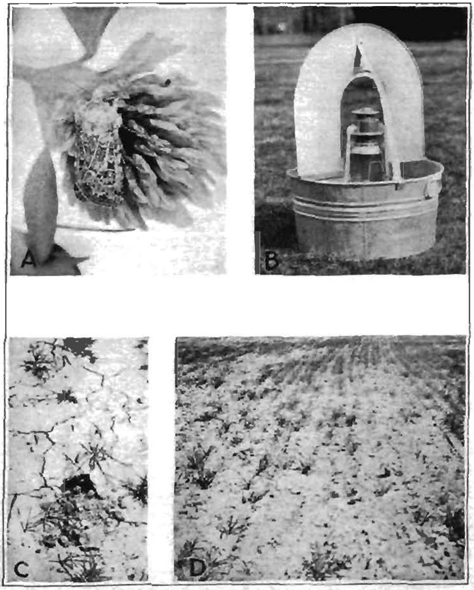
A.—Cast head of seventh-instar larva.

B.—Cast head of eighth-instar larva with setæ numbered. *Adf.*, adfrontal sclerite, *adf. i*, *adf. ii*, its setæ; *fr.*, frontal sclerite; *fr. i*, frontal setæ; *fr. o*, frontal puncture; *cl.*, clypeus; *cl. i*, *cl. ii*, its setæ; *lbr.*, labrum; *ant.*, antennæ; *md.*, mandible; *md. i*, *md. ii*, its setæ; *i* to *xi*, setæ of epicranium. *I* to *IV*, first four ocelli.

PLATE 30

*Porosagrotis orthogonia:*

A.—Moth feeding on clover blossom.
B.—Light trap.
C.—Excavation made by horned lark in digging out cutworm.
D.—Wheat field attacked by the larvæ.


A
B
C
D

# BIOLOGY OF EMBAPHION MURICATUM

By J. S. WADE, *Scientific Assistant, Cereal and Forage Insect Investigations, Bureau of Entomology;* systematic description of the larva by ADAM G. BÖVING, *Bureau of Entomology, United States Department of Agriculture*

## INTRODUCTION

Considerable damage has been wrought during the past six or seven years by the larvæ of *Embaphion muricatum* Say and related species of false wireworms to growing wheat and other field crops throughout the semiarid and middle western United States. The area of greatest injury embraces approximately the western half of Nebraska, Kansas, and Oklahoma, and the eastern third of Colorado and New Mexico, although losses of varying magnitude have been reported in various localities over the greater part of all these States. In view of the obscure character of the injury, it seems quite probable that much crop damage commonly charged to other causes in reality has been brought about by this pest. The steady transformation in recent years of grassy prairies into cultivated fields has been an important factor, because the removal of native food plants causes this and related species to feed more and more upon cultivated grains. The hardiness of the insect and the rapidity of its adaptation to changed conditions and to new host plants indicate that this species is potentially a serious menace to grain production within the infested region.

## EARLY RECORDS

The species under discussion was originally described in 1824 as *Akis muricata* by Thomas Say (*1*),[1] who stated that it—

inhabits Arkansa at the Rocky Mountains

and that—

as it does not entirely agree with any genus the characters of which Latreille has noted, it may be proper to remove it to the Blapsidae, under a separate genus, which may be named *Embaphion*.

This description was reprinted in 1859 in the LeConte edition of Say's (*1*) works, with a brief supplementary editorial note indicating relationship of the genus Embaphion to the genus Eleodes. A single specimen from Texas was described as *Eleodes contusum* by LeConte (*2*) in 1853, who stated that it—

resembles *E. muricatum* Say but is longer and narrower with the broad margin of the elytra more suddenly reflexed and almost perpendicular. Although so different in form, this genus is only distinguished from *Eleodes* by the inferior plane of the

[1] Reference is made by number (italic) to "Literature cited," p. 334.

mentum being more rounded and more deeply impressed; its anterior margin is slightly incised, the lateral angles are so much reflexed as to be invisible; the tarsi are sulcate beneath and fringed at the apex and sides with short spines; the middle joints of the posterior tarsi appear more elongated than in *Eleodes*. I have grave doubt of the generic value of any of these differences, and several nondescript species from New Mexico seem to be intermediate both by the form of the body and by the differences in the mentum.

The characters distinguishing *Embaphion contusum* from *Embaphion muricatum* were discussed by LeConte (*3*) in 1859. A brief résumé of the previous history of the genus was made by Lacordaire (*4, v. 5, p. 152; atlas, pl. 50, fig. 2*) in 1859, in which attention was called to Say's inadequate designation of the genus *Embaphion* and to variation of the species with its geographical distribution. Horn (*5, p. 320–322*) in 1870, in his discussion of the genus, indicated the feeble taxonomic characters which separate this genus from Eleodes. In referring to the species *Embaphion muricatum* he states:

This species may be readily distinguished from the others of the genus by the very broad foliaceous margin of the thorax and elytra, very strongly reflexed. The elytral margin extends beyond the apex and the two meet on a line with the suture. The thoracic margin is broad and widens behind, so that the hind angles are prominent, sub-acute, and project backwards over the basal angles of the elytra. The thorax itself (less the margins) is narrow, longer than broad, and about equal to half the width of the elytra (without margin). The disc of elytra (without margin) is elongate oval, the humeral angles not prominent and are rounded. The angles formed by the margin are nearly right. The base of the thorax is strongly trisinuate; the base of the thorax proper being rounded, that of the margin on each side emarginate. The base of elytra is emarginate at middle, and on each side broadly rounded.

He stated further that *Embaphion concavum*, described by LeConte (*2*) in 1853, is

merely a large form with more strongly reflexed margins. The elytra of both forms are sculptured with approximate series of fine punctures, each bearing a short hair.

Blaisdell's (*11, p. 473–477*) very full discussion (1909) of the adult forms of the species and their taxonomic relationships leaves little to be desired. He especially emphasizes the salient type characters.

Margins of the thorax and elytra broadly foliaceous and strongly reflexed, basal angles of the prothoracic margins projecting strongly backward over the basal angles of the elytra.

DISTRIBUTION

Nebraska: Alliance, altitude 3,971 feet, August, H. F. Wickham; Beaver City, altitude 2,150 feet, M. H. Swenk (*12*), September, J. S. Wade; "Nebraska," May to August, H. F. Wickham (*8*).

New Mexico: Chico, altitude 6,882 feet, September, D. J. Caffrey; Clovis, August, H. F. Wickham; Koehler, altitude 5,500 feet, June, V. L. Wildermuth, August, W. R. Walton; Vaughn, September, H. F. Wickham; Maxwell, altitude 5,894 feet, D. J. Caffrey; Willard, altitude 6,091 feet, H. F. Wickham.

Kansas: Clark County, altitude 1,962 feet, June, F. H. Snow (*10*); Colby, altitude 3,150 feet, August, J. S. Wade; Dodge City, altitude 2,509 feet, August, J. S. Wade; Hamilton County, altitude 3,000 feet, F. H. Snow (*10*); Liberal, altitude 2,839 feet,

July, J. S. Wade; Meade, altitude 2,503 feet, July, J. S. Wade; Morton County, altitude 3,000 feet, F. H. Snow (*10*); Norton, altitude 2,284 feet, August, J. S. Wade; Rice County, June, H. F. Wickham; Scott City, altitude 2,971 feet, August, J. S. Wade; Wallace County, altitude 3,000 feet, F. H. Snow (7); Wellington, altitude 1,205 feet, July, J. S. Wade; "Kansas to Texas," G. H. Horn (5). "Western Kansas: In Arkansas and Smoky Hill Valleys," E. A. Popenoe (*6*).

North Dakota: Dickinson, altitude 2,411 feet, August, H. F. Wickham; "Dakota," W. G. Dietz; "Dakota," May to August, H. F. Wickham (*8*).

Colorado: Bellevue, altitude 8,993, H. F. Wickham (*9*); Colorado Springs, altitude 6,072 feet, H. F. Wickham (*9*); Denver, altitude 5,279 feet, April, H. Soltau; Greeley, altitude 4,652 feet, June, H. F. Wickham; Fort Collins, altitude 4,994 feet, H. F. Wickham (*9*); LaSalle, altitude 4,676 feet, September, H. F. Wickham; Pueblo, altitude 4,685 feet, October, H. Soltau; West Las Animas, H. F. Wickham (*9*); "Colorado," May to August, H. F. Wickham (8).

Texas: Amarillo, altitude 3,676 feet, August, H. F. Wickham; Canadian, altitude 2,340 feet, August, H. F. Wickham; Mobeetc, July, H. S. Barber; Texline, altitude 4,694 feet, September, I. R. Crawford.

Montana: Assinniboine Mountains, Hubbard and Schwarz, "Montana," May to August, H. F. Wickham (8).

South Dakota: Alexandria, altitude 1,354 feet.

Mexico: Nuevo Laredo, Tamaulipas, Hoge.

## INJURY

The principal damage caused by these insects is that wrought by the larvæ during the fall in devouring recently sown or newly sprouted wheat grains shortly after the seed wheat has been drilled. These larvæ often may be found in large numbers in infested fields at such periods working steadily along through the soft soil of the drill rows, either wholly devouring or destroying for germination purposes every wheat grain within a drill row for many yards. Within the region of greatest infestation the principal injury is done between September 20 and October 15. The injury to the grain is characteristic of this family. Sometimes the entire contents of the grain are removed, leaving all or part of the shriveled outer husk; in some cases the ends of the grain are nibbled away or portions of the ventral crease are neatly furrowed out. The adults also are known to feed upon wheat grains and other seeds, being present around the bases of wheat stacks in July, where they may be found tearing away the spikelets of grain in newly cut wheat heads to devour the kernel within, or they may be found feeding upon the scattered grains. The extent of the injury varies annually in accordance with seasonal conditions, little or no damage being done in localities where an abundance of rainfall occurs, and where temperature and other factors are favorable to growing crops, whereas at the same time considerable loss may be experienced in other localities, varying from 10 to 50 per cent or more of the wheat of an entire neighborhood, where weather and other conditions render normal development of this crop impossible. In view of the fact that the larvæ of this pest usually may be found working with those of other nearly related species of true and false wireworms, it becomes increasingly difficult to isolate and estimate singly the exact amount of injury wrought by this particular pest.

## HABITS

The larvæ are exceedingly active and quick and, if exposed to light by the plow or otherwise disturbed, have the power of wriggling very quickly down out of sight into the soil. They are also occasionally found upon the surface of the ground feeding upon seeds of weeds and of other plants, in spots where the soil may be slightly moist and where they are covered by wheat shocks or by matted masses of dried Russian thistles or other weeds. While they appear to prefer habitats where there may be a slight degree of moisture, such as moist, poorly drained spots in fields, and cool, damp cellars, yet they do not live long in thoroughly wet soil. Both larvæ and adults often may be found in numbers beneath dried weeds along irrigation canals. The larvæ habitually feed during warm weather at a depth varying from 2 to 5 inches, according to condition of the soil. As they burrow from place to place, they feed upon the roots and seeds of plants, and possibly to a certain extent upon organic matter where this is sufficiently decayed. When placed under artificial conditions the larvæ feed readily not only upon germinating wheat, but upon corn and roots of grasses. They are cannibalistic in that they feed upon other larvæ of the same species which die or become weakened because of injury or disease. They also feed upon their own exuviæ.

The adults, in common with those of other nearly related species, are very hardy and active and appear to be able to withstand considerable variations of temperature. While they, like the larvæ, appear to prefer cool, moist spots, they do not survive temperatures as low as $-9°$ F. They have been collected in August beneath wheat shocks in fields where the temperature was as high as 100°. The adults easily climb all over wheat where standing or in the stack or shock, and they burrow with apparent ease far into the piles of unthrashed grain. They are also frequently found in the burrows of small mammals. During periods of prolonged drought the beetles may seem to have entirely disappeared, yet immediately following a shower or rainstorm, curiously enough they reappear in large numbers, where previously none could be found.

## DESCRIPTIONS

### EGG

Size slightly variable, being 1.1 to 1.3 mm. in length and 0.60 to 0.62 mm. in width; shape circular in cross section and oval in longitudinal section; without sculpturing; color pure white when first deposited, changing to yellowing brown before hatching.

### MATURE LARVA[1]

Length 27 mm.; color testaceous with head and legs somewhat darker; anterior and posterior margins of prothorax and posterior margins of the following segments castaneous-testaceous. Surface corneous. Form elongately cylindrical, more than

[1] Description and Plates 31 and 32 by Adam G. Böving.

10 times longer than wide; dorsally very convex, ventrally flattened; pygidium movable in the directions up and down, subconical, obtusely pointed. Head, ventral sides of the thoracic segments and of the first abdominal segment, legs, and pygidium (Pl. 32, C) clothed with rigid or soft setæ; rest of body glabrous with very few and small ventral hairs.

Cranium (Pl. 31, B) rounded, nutant, exserted, one-third broader than long (from epistomal margin (*epi*) to foramen occipitale), broadest medianly, dorsally somewhat convex. Anterior frontal angle (*fa*) low and rounded. Frons (*f*) three-fourths the length of cranium, about as long as wide with extreme width anteriorly, side margin convex. Epicranial halves (*epc*) meeting dorsally; epicranial suture one-fourth the length of cranium; ventrally (Pl. 31, E) the halves are separated by gula (*gu*); dorsally with a few, laterally and ventrally with many hairs. Gula and submentum (*sm*) both distinct, coriaceous. Gula almost square, with tentorial pits (*tp*) at the middle of the side margins. Submentum trapezoidal, broadest posteriorly; side margins slightly concave and adjacent to maxillary articulating area. Clypeus (*cl*, Pl. 31, B) trapezoidal, widest behind, length to extreme width as one to four, medianly with slight transverse deepening, set on each side with one minute seta near the middle line and two well-developed setæ near the lateral margin. Labrum well-developed, movable, transversely rectangular, length to width as one to three, anterior margin almost straight, anterior corners rounded; disk on each half with a median transverse series of five large setæ, and an anterior series of three long, thin, and straight setæ; right behind those but on the ventral side of labrum another series of four shorter, stronger, and curved setæ. Epipharynx (*eph*, Pl. 31, A) forming the buccal surface of labrum, soft-skinned with posterior transverse, broad, sinuous, chitinous band, that carries one pair of stublike sharp teeth; on the soft-skinned part anteriorly to these teeth a pair of tiny hooks; near anterior margin scattered minute setæ and ring-shaped punctures. Just behind antenna two ophthalmic spots, both transverse, slightly posteriorly convex, the anterior a little more external and about three times longer than the posterior; immediately in front of the anterior are numerous setæ; the ophthalmic spots are likely to disappear in full-grown larvæ. Antenna (Pl. 31, B) closely behind the mandible, attached in articular cavity with distinct border; basal antennal membrane well developed; basal article cylindrical, about as long as epicranial suture, second article as long as basal article, more clavate, apical article very small, conical, papilliform, carrying one seta; no supplementary appendix besides the apical article. Mandibles (Pl. 31, F) of right and left side differing in shape; both apically bifid ($a^1$, $a^2$); both with one tooth (*t*) between apex and molar part (*m*); tooth of right mandible, however, prominent and placed near apex, that of left mandible less developed and placed closer to molar part; molar part of right mandible with bituberculate crown, that of left mandible with hollow crown; ventrally (Pl. 31, D) with cutting part deeply excavated; exterior surface ("the back of the mandible") distally with a slightly carinate margin (Pl. 31, F, *c*), proximally with a soft-skinned, whitish swelling (*s*) from an excavation (*e*) opposite the molar part; three to four strong setæ from the anterior portion of the swelling, two from the posterior, several small, soft setæ near dorsal mandibular articulation. Maxilla dorsally completely covered by mandible; palp (Pl. 31, E) surmounting mala (*ma*) (maxillary lobe) with one-third of its own length; palpiger (*pag*) small, ring-shaped; basal article about as wide as long, second article cylindrical, somewhat narrower and more than twice as long as basal article, apical article two-thirds as long and half as thick as the second, conical, with soft tip; each article with one or two thin setæ; mala (*ma*) on buccal surface (Pl. 32, F) with two series of well-developed, somewhat curved setæ; base of stipes (Pl. 31, E, *bs*) (that is, region where stipes and cardo meet) rather short; proximal half of inner margin of stipes ($is_1$) connected with exterior half of maxillary articulating area ($ar^1$), distal half ($is_2$) right behind mala, free; ventral stipital surface with several strong setæ; other setæ on the exterior surface; cardo as long as

exterior margin of stipes, adjacent to slightly curved hypostomal thickening (*hyp*) between fossa for ventral mandibular condyle (*fm*) and fossa for tip of cardo (*fc*); inner margin of cardo connected like stipes with exterior half ($ar^{I}$) of maxillary articulating area. Maxillary articulating area protuberant, soft, divided into two halves; exterior half ($ar^{I}$) connected with maxilla, subdivided into an upper and lower portion; interior half ($ar^{II}$) connected with submentum, entire; no setæ. Mentum (*me*) almost square, side margins free; on each side about five setæ of different length. The two stipites labii (*stla*) fused into a slightly chitinized unit, carrying on each side two setæ; labial palp about half as long and half as thick as maxillary palp; basal and apical articles slightly different in length, basal article somewhat clavate, apical article conical and half as thick as basal article; ligula (*li*) small, narrow conical, with one terminal pair of setæ. Hypopharyngeal sclerite (Pl. 32, A, G, H, *hsc*) elongate rectangular, projecting, strong; anteriorly tricuspidate with median cusp largest; disk somewhat excavate with a posterior semiglobular tubercle; molar part of mandible and hypopharyngeal sclerite grinding together (Pl. 31, D, F, G). The hypopharyngeal bracon (Pl. 32, A, G, H, *hbr*) is well developed as a chitinous rod in the buccal membrane between the ventral mandibular articulation and the hypopharyngeal region. Prothoracic legs (Pl. 31, C, H, I; 32, B) considerably stronger than the mesothoracic and metathoracic ones and with coxæ attached so closely together that they almost touch each other at base. Coxa of first pair about as long as wide; many fine, scattered hairs on exterior and interior surfaces; trochanter about as long as coxa, on the inner side (Pl. 31, H) distally with two spinelike setæ and also with a few other thin hairs; femur (*fe*) about as long and wide as trochanter, armed with five spinelike setæ, also with many thin, scattered hairs; tibia (*ti*) about twice as long as thick, almost same length as femur but not fully as wide, armed with five spinelike setæ and also with fine, scattered hairs; tarsus (*ta*) of almost same length as tibia, claw-shaped, strong, but rather slender, with backward-facing surface distally excavate and proximally carrying a round soft-skinned region around a short but strong seta; another and similar seta set close to it at the end of the excavation; otherwise no setæ or hairs on tarsus. Second and third pairs of legs inserted farther apart than the first pair; the arrangement of their setæ very similar to that of the first pair, but the proportional sizes between the articles somewhat different from those of the first pair. Prothoracic eusternum (Pl. 32, B, *eu*) large, rhomboidal, anteriorly almost reaching the front margin of the segment, only separated from this margin by a small presternal area (*y*); the hypopleural chitinization ($h_{I}$ and $h_{II}$), and especially its prehypopleural part ($h_{I}$), large and strong; prothoracic tergal shield subquadrate, slightly wider than long, with anterior and posterior margins, as mentioned above, darker than the rest of the shield and finely longitudinally striated. Mesothorax and metathorax with transverse, subtriangular, narrow presternum (*y*), laterally adjacent to poststernellum (*z*) of the preceding segment; hypopleural chitinizations ($h_{I}$ and $h_{II}$) well developed, but considerably smaller than those of prothorax; poststernellum of metathorax not present, preepipleurum of mesothorax ($e_{I}$) subtriangular, carrying first thoracic spiracle; preepipleurum of metathorax not distinctly limited, carrying the rudimentary second thoracic spiracle; postepipleurum ($e_{II}$) of both segments well developed, more or less fused with the corresponding preepipleura; mesothoracic and metathoracic tergal shields transversal, subrectangular, about twice as wide as long, right behind anterior margin with a dark transverse line; posterior margin darker than rest of segment, finely longitudinally striated. Typical abdominal segment (that is, one of the eight anterior abdominal segments) with fused sternal and hypopleural areas (*ster*), covered by a single, longitudinally rectangular shield, which posteriorly has a rather dark, transverse, longitudinally finely striated margin; one seta present near the anterior and one seta near the posterior margin; additionally the sternum of first abdominal segment is anteriorly densely

set with small, soft setæ; similar outfit lacking on the other abdominal segments. Epipleural region narrow. Tergal region with a dark line above the spiracle. Tergal shield (*ter*) single, posteriorly with a dark, longitudinally striated margin. Anterior abdominal segments transverse, slightly wider than long; sixth, seventh, and eighth abdominal segments subquadrate. Ninth abdominal segment smaller than the preceding segment; dorsal part or pygidium pointing upwards, subconically produced, above somewhat flattened, below broadly convex, apex obtuse, laterally with margin set with a single series of strong, short setæ, whole surface with scattered, fine setæ; ventral part of ninth segment small, transverse, soft. Tenth abdominal segment (or "anal segment") small, with trilobate upper transverse anal lip, with a pair of conical and, except at the tip, setose ambulatory warts, laterally to anus a small triangular lower lip. Spiracles (Pl. 32, I) annular, shortly oval, transversely placed; opening at the bottom of cup-shaped peritrema, linear, unprotected by hairs. The number and development of setæ on the first pair of legs vary according to species and do not offer any generic character. The same is the case with the setal arrangement of pygidium.

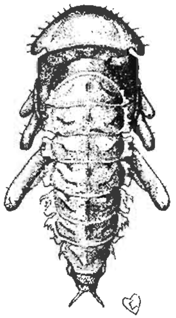


Fig. 1.—Pupa of *Embaphion muricatum*, dorsal view.

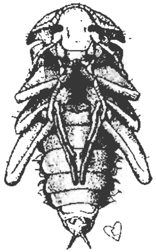


Fig. 2.—Pupa of *Embaphion muricatum*, ventral view.

PUPA (FIG. 1, 2)

Length 11 mm. Width 5.6 mm. Free. Arcuate. Color pinkish white, with ferruginous tinge on pronotum. Femora and tarsi fuscate, other appendages partly translucent. Pupa becomes more strongly colored immediately before issuance of adult. Head pressed to prosternum. Pronotum broad and projecting somewhat anterior to head, making the head nearly invisible from above. Frons impressed. Vertex prominent. Antennæ placed backward near sides of prothorax. Mesonotum narrow. Legs not pressed against body. Tips of wing cases extending to the anterior margin of metanotum. Second to fifth abdominal segments bearing on each side of tergites flat, lacerated protuberances, obtuse, pointed and directed posteriorly. Eighth segment ending in two slightly divergent, acute processes.

ADULT (FIG. 3) [1]

Oval to oblong-oval, brownish to piceous black, thoracic and elytral margins very broad and foliaceous, strongly reflexed.

*Head* small, less than twice as wide as long, plane, sides of the frons slightly prominent, punctate, punctures very feebly subasperate, fine, not dense, each with a small curved and short seta, frontal suture usually not visible. *Antennæ* rather long, quite slender, outer four joints very slightly compressed and scarcely widened, third joint shorter than the next two taken together, fourth scarcely longer than the fifth, the latter and sixth subequal, seventh shorter, eighth feebly shorter than the seventh and slightly triangular, ninth and tenth suborbicular, eleventh subovate.

*Pronotum* with margins very broadly foliaceous, the margin more than one-half wider than the *disc*, the latter comparatively narrow, longer than wide at middle, very feebly convex, usually with irregular depressed areas; finely, more or less subasperately and sparsely punctate; reflexed margins wider posteriorly and more or less concave, a little more distinctly punctate, punctures less sparse, each with a short curved seta; *apex* deeply and feebly subquadrately emarginate, the emargination about one-half wider than deep, sides almost parallel, and scarcely margined; *sides* evenly but not strongly arcuate, moderately converging from base to apex; *base* proper feebly arcuate, not margined and about equal to the length, laterally sinuate; apical angles rather narrowly rounded and formed by the advanced foliaceous margins and nearly as long as the head; basal angles are posteriorly prominent, subacute, and projecting backward over the basal angles of the elytra.

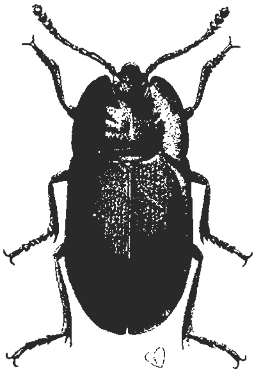


FIG. 3.—Adult of *Embaphion muricatum*, dorsal view.

*Propleuræ* smooth and impunctate; inferior surface of the foliaceous margins obsoletely punctate.

*Elytra* oval to elongate oval; *margins* broad and reflexed, angles at humeri nearly rectangular and more or less truncate at base, posteriorly extending beyond the apex, the two meeting on a line with the suture above the true elytral apex, and defined from the same by a slight groove, borders evenly arcuate from base to apex or more or less parallel basally; *base* evenly but not strongly emarginate; *humeri* proper broadly rounded and not prominent; *sides* proper more or less evenly arcuate; *apex* proper not produced and narrowly rounded; *disc* plane, feebly convex, at times slightly concave, the inflexed sides nearly straight and oblique, gradually and not strongly arcuately declivous behind; *surface* sculptured with approximate series of fine asperate punctures, which become more irregular and slightly denser laterally. Each puncture bears a short and rather robust curved seta.

*Epipleuræ* narrow, not attaining the humeral margin and not dilated, but gradually narrowing to apex, not defined from the inflexed sides of the elytra, and on the same plane; superior margin obsolete, except near apex; elsewhere represented by a line of punctures or a faint groove.

[1] Reprinted from F. E. Blaisdell (*11, p. 473-476*).

*Sterna* more or less dull, finely and not distinctly sculptured.

*Parapleuræ* smooth, rather sparsely but not very distinctly punctate.

*Abdomen* horizontal, very finely and sparsely punctulate, obsoletely rugulose and quite evenly convex.

*Legs* rather slender, moderate in length. Anterior femora mutic, protibial spurs similar in the sexes, the anterior slightly longer than the posterior. Protarsi simple.

## LIFE HISTORY AND DEVELOPMENT

The principal observations on the life history and development of *Embaphion muricatum*, as given below, were made under laboratory and field conditions in south-central Kansas, at an altitude of approximately 1,200 feet. Under different conditions of latitude, altitude, and humidity there would doubtless be found more or less marked variations. The records are unfortunately based on incomplete studies for, owing to working conditions and to pressure of other duties, there was no opportunity to conduct a sufficiently extensive series of experiments to render all observations conclusive.

The eggs are deposited in loose, dry, or slightly moist soil at a depth of ½ to 1 inch, sometimes singly, but more often in clusters of two or three to a dozen or more eggs at one place. At temperatures ranging from 80° to 90° F. the average period of incubation is approximately 10 days, whereas at temperatures of 68° to 70° F. the egg stage is approximately 13 days. Undoubtedly weather conditions and the time of year have a direct bearing on the duration of the egg period.

During the later stages of development and shortly before hatching, the surface of the egg becomes light brown in color, and the shell appears to expand slightly and to become more flexible, while the movements of the young larva can be noted within. During the process of hatching, the struggles and the lifting pressure of the young larva burst the shell and the larva emerges by rather slow periodic movements, as its integument is very soft and fragile. Though the young larva often remains for some time near the place of hatching, yet it is capable of locomotion soon after emergence. All normal eggs of the same egg cluster usually hatch within a short period, generally a few hours. While abnormal weather conditions may prolong the period of hatching, no injurious effects of such retardation are noted in the eggs. No infertile eggs were ever collected under field conditions. Soon after the emergence of the larvæ the empty eggshells become more and more contracted and dried up, until eventually only tiny, shrivelled fragments remain.

Upon hatching the larva averages 3.5 to 3.75 mm. in length and is yellowish white. The color changes slightly after each molt until at maturity the larva becomes a deep yellow.

The length of the larva stage, according to an experiment consisting of 31 larvæ hatched in June and kept in a cool cellar at an average temperature of 68° F., averaged for the survivors 79 days, while in an experiment consisting of 49 larvæ, under similar conditions, it varied from 76 to

96 days, though the average duration was 85 days. The larvæ as hatched were placed in small tin salve boxes containing about ½ inch of slightly moist soil and split wheat grains. As the larvæ became large, whole wheat grains were used as food. Under field conditions many of the larvæ appear to become nearly mature during late fall and overwinter in this condition. From about November 1 to March 15 in the latitude of southern Kansas they are exceedingly inactive and feed but little. The rapidity of growth of the larvæ undoubtedly depends to a large degree upon weather and seasonal conditions and the quantity and quality of food available. Shortly before the period of pupation the larva does not feed and assumes a semidormant stage of approximately 7 to 9 days' duration.

The pupa stage, when rearings were conducted under laboratory conditions, comprised 18 to 20 days. The pupæ are pinkish white immediately after transformation, and as development proceeds the color changes to light yellow. Shortly before the adults emerge the appendages take on a yellowish brown tint.

The newly issued adults are of a brighter color, and the chitinous portions of the body are soft. Within a few days, however, the color darkens and the integument hardens so that the newly emerged adults are not distinguishable. Under artificial conditions mating does not become general for a week or more after emergence. Oviposition and feeding appear to occur usually at night. The adults are crepuscular. They may be found abroad in greatest numbers on cloudy days or in early morning or late evening. On clear days, during the middle or warmer portion of the day, they remain under shelter. While usually inactive at such periods, if disturbed they will run with great rapidity. The insect may overwinter both in the adult and in the larva stages. In the latitude of southern Kansas, however, the mortality of such overwintering adults is great.

## ENEMIES

While the incomplete character of the life-history work performed with *Embaphion muricatum* afforded comparatively little opportunity for obtaining parasites under artificial conditions, or for obtaining data on other enemies for possible use in control work, yet some noteworthy information was obtained. From adults of *E. muricatum* collected by the writer from barley at Colby, Kans., on August 25 there were reared on October 23 adults of a parasite determined by A. B. Gahan of the Bureau of Entomology as *Perilitus eleodis* Viereck (*13*). No life-history work on these parasites was attempted.

Considerable difficulty was experienced in rearing larvæ owing to the presence in the cages of a fungus, *Metarrhizium anisoplae* Metsch. Soc.; and though the apparatus and soil were sterilized, yet the mortality was sufficient at times to interfere to a marked extent with the rearing. A

number of the larvæ in the cages were also attacked by an obscure bacterial disease. This appeared to be identical with that described by Prof. Swenk (*12*). There would appear somewhere upon the body sutures small circular or irregularly shaped dark brown spots, and these, after a few days, would become larger, until in some instances they would cover one-third to one-half of the body surface. This disease usually caused the death of the larvæ within varying periods of time. Larvæ found under normal field conditions are sometimes found to be affected both with Metarrhizium and with the disease.

## CONTROL

While no extended series of experiments relative to control of the insect thus far has been found possible, yet the information secured on the subject has been sufficient to assure the practical value of the measures here recommended in reducing or preventing damage.

A systematic rotation of crops is one of the most effective procedures in cutting down damage. The maximum injury always may be found upon those areas where the ground has been cropped to wheat continuously for several years, whereas the minimum injury is found where corn, kafir, milo, and other crops are grown which require some degree of cultivation during the growing season. An important factor in migration and infestation lies in the fact that the beetles are wingless and therefore become dispersed much more slowly than do winged forms.

A number of fields within infested areas, which were also infested by the corn earworm (*Chloridea obsoleta* Fab.) and other insects of somewhat similar habits, were plowed by farmers during late fall or early spring to destroy the pupæ, and it was found that such measures were of considerable value in control of the false wireworms in the soil. The pupal cells were crushed and the pupæ buried or thrown out upon the surface, where they were exposed to the elements and to predatory enemies.

It is not only good farm practice but also advantageous as a control measure to destroy and remove from infested fields and adjacent fence rows all clumps of Russian thistles and other weeds or heavy growths of grasses likely to shelter these beetles.

While adults in small numbers are known to feed upon poisoned bran mash and similar preparations, experiments in poisoning the larvæ were not satisfactory. It appears doubtful that such poisoning will ever prove of practical value in dealing with this pest.

Late sowing of wheat in the fall also has been tried as a possible control measure, but does not appear to be successful unless the season is a very dry one, and even then if the seed has to lie in the ground any appreciable length of time before rain and germination much injury is likely to result, for the larvæ are most active in the dry, loose soil under such conditions.

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## PLATE 31

*Embaphion muricatum.*

A.—Epipharynx (*eph*) and anterior margin of labrum.

B.—Head: *cl.*, clypeus; *fa*, anterior angle of front; *epi*, epistoma; *f*, frons; *epc*, epicranium.

C.—Lateral view of larva.

D.—Mandibles and hypopharyngeal sclerite from below. Concavity of molar part of left mandible grinding against the sclerite.

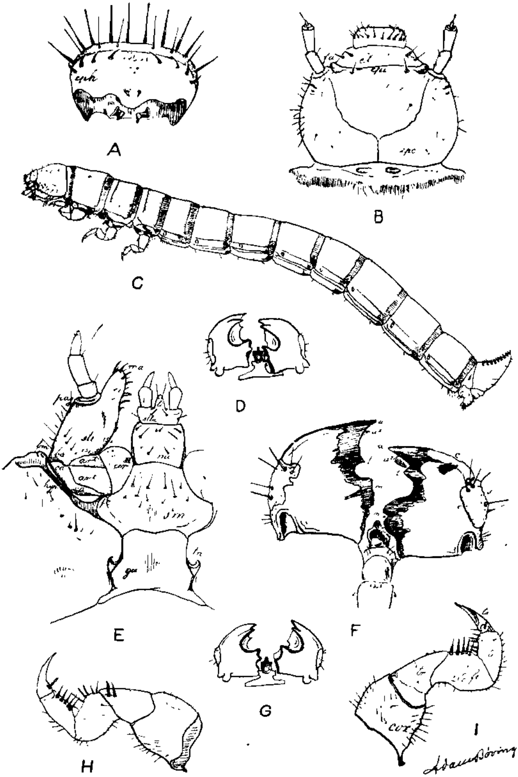
E.—Gula, labium, and right maxilla from ventral side: *gu*, gula; *tp*, tentorial pit; *sm*, submentum; *me*, mentum; *stla*, stipes labii; *li*, ligula; *hyp*, hypostoma; *fm*, fossa for mandible; *fc*, fossa for cardo; *ar*, maxillary articulating area; *ca*, cardo; *sti*, stipes maxillaris; *bs*, basis of stipes; $is_1$ and $is_2$, inner margin of stipes; *ma*, mala maxillaris (probably lacinia); *pag*, basal membrane of maxillary palp.

F.—Dorsal side of right and left mandible, hypopharyngeal sclerite between them: $a^1$ and $a^2$, the bicuspidate mandibular apex; *t*, tooth of cutting edge; *m*, molar part; *c*, carinate edge on exterior side of cutting part of mandible; *s*, soft-skinned, seta-bearing elevation below the carinate edge; *e*, margin of chitin framing the soft elevation.

G.—Mandibles and hypopharyngeal sclerite from below; no grinding in this position.

H.—Left anterior leg showing the anterior face of the leg hanging perpendicularly down from a horizontally placed larva.

I.—Left anterior leg, exhibiting its posterior face: *cox*, coxa; *tr.*, trochanter; *fe*, femur; *ti*, tibia; *ta*, claw-shaped tarsus, shortly but not correctly designated as "claw."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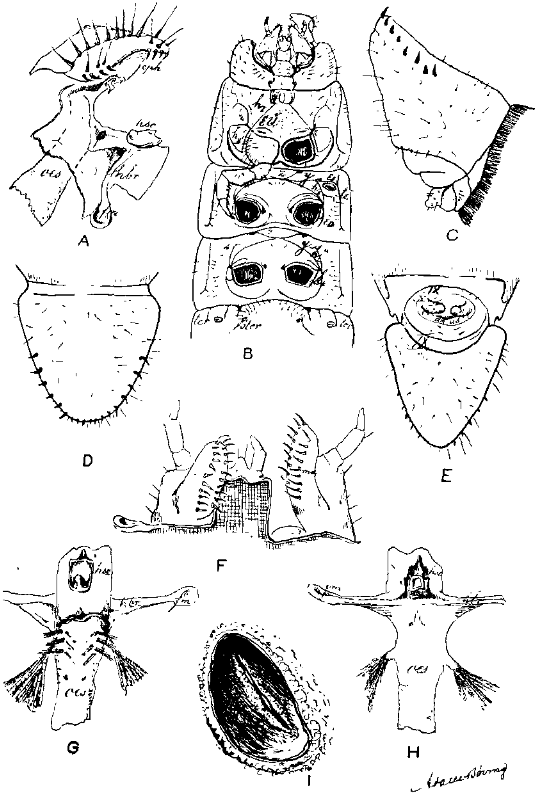
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## PLATE 32

*Embaphion muricatum:*

A.—Lateral view of buccal cavity with mouthparts removed: *eph*, epipharynx; *hsc*, hypopharyngeal sclerite; *hbr*, hypopharyngeal bracon; *fm*, fossa of ventral condyle of mandible; *oes*, oesophagus (note the distance between sclerite and entrance to oesophagus).

B.—Ventral view of head, the thoracic segments, and the anterior portion of first abdominal segment: *y*, presternum; *eu*, eusternum (Snodgrass) or basisternum (Crampton); $h_I$, prehypopleurum; $h_{II}$, posthypopleurum; *s*, poststernellum (presternum and poststernellum constitute together the ventral intersegmental region); $e_I$, preepipleurum; $e_{II}$, postepipleurum; *te*, tergite; *ster*, sternal shield of abdominal segments; *ep*, abdominal epipleurum; *ter*, abdominal tergite.

C.—Pygidium, lateral view.

D.—Pygidium, dorsal view.

E.—Pygidium, ventral view; IX, ninth abdominal ("pygidial") segment; X, tenth abdominal ("anal") segment.

F.—Maxillæ, ligula, labial palpi seen from the buccal cavity. (Hypopharyngeal region removed.)

G.—Hypopharyngeal region, oesophagus, and hypopharyngeal bracon which all were removed from figure F: *hsc*, hypopharyngeal sclerite; *hbr*, hypopharyngeal bracon; *fm*, mandibular ventral fossa; *oes*, oesophagus.

H.—Hypopharyngeal region, same piece as figure G, reversed: *hsc*, base from which hypopharyngeal sclerite originates; *hbr*, hypopharyngeal bracon; *fm*, mandibular ventral fossa; *oes*, oesophagus.

I.—First thoracic spiracle.

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## GENETIC BEHAVIOR OF THE SPELT FORM IN CROSSES BETWEEN TRITICUM SPELTA AND TRITICUM SATIVUM [1]

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### INTRODUCTION

In connection with genetic studies of density in the wheat spike, it was noted that the appearance of different specific forms in certain wheat crosses introduced marked irregularities in density curves of the second and following generations, and that the densities of *Triticum sativum* Lam., *T. polonicum* Linn., *T. spelta* Linn., etc., were affected in different degrees when a certain known density factor was introduced through hybridization. In some instances there were partial and sometimes total inhibitory effects in regard to density, depending upon the subspecies and also the kind of density factor involved in the cross.

The occurrence of these irregularities which appeared to be caused by the spelt character in some crosses led to the study of the nature and genetics of the species *T. spelta*. Although studies have been made of the mode of inheritance of the spelt form in a large number of interspecific crosses, only the different modes of inheritance in crosses where the parents are *spelta* and *sativum* are presented in this paper. In other crosses, such as *turgidum* Linn. × *sativum*, *durum* × *sativum*, *dicoccum* Schr. × *sativum*, etc., spelts invariably appear in the $F_2$ generation. The mode of inheritance of these spelt forms is complex and variable, so their discussion here has been omitted.

The plants on which these studies were made were grown, with a few exceptions, on Arlington Farm, near Washington, D. C., or on the Plant Introduction Station, Chico, Calif., both operated by the United States Department of Agriculture. The crosses were made at the former place in 1913.

[1] The specific name *T. sativum* as used in this paper refers only to the forms *T. vulgare* Vill., *T. compactum* Host., and *T. capitatum* Schlz. These three forms are essentially the same species, their differences being merely a question of internode length. The word wheat is frequently used as an English designation for these forms, and when so used does not include such other forms as *T. durum* Desf., *T. polonicum*, etc.

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## SPECIFIC DIFFERENCES BETWEEN TRITICUM SATIVUM AND TRITICUM SPELTA

The shape of the outer or sterile glume is an important character in the differentiation of wheat species. The glume of the true *T. sativum* form (fig. 1, B) is, as a rule, soft, with a somewhat pointed apex. It is rarely and very weakly keeled along the entire length. About 0.5 to 1 mm.

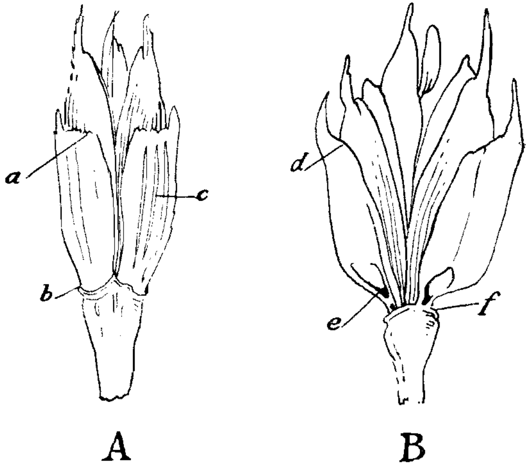


FIG. 1.—Characteristic spikelets of *Triticum spelta* (A) and *Triticum sativum* (B). Note upright and tight position of the glumes of *spelta* and the loose, spreading habit of *sativum*. *Spelta* has a flat shoulder (*a*) with two or three indentations, the shoulder of *sativum* (*d*), though varying widely, is generally not so prominent. It is often rather tapering with no indentations. The base of the sterile glume of *spelta* (*b*) is broad, showing firm attachment to the rachis. The glumes can not be opened without breaking them at the base. In *sativum* the base (*f*) is narrow and is weakly attached to the rachis, and the glumes can be opened easily. There are one or more depressions (*e*) at the base of the glume in *sativum* which are not present in *spelta*. The glume of *spelta* is more or less uniform in width. It is stiff and has prominent corrugations (*c*); that of *sativum* is narrow at base, widens, then tapers again gradually. It is very thin and soft, and the veins on the glumes are not so prominent. *Sativum* has a weak keel while *spelta* has a very strong one. The *Spelta* spikelet usually develops only two kernels, *sativum* often develops three or more.

above the point of attachment there is a wrinkle or depression. The base is rather narrow and is very weakly attached to the rachis. The glume characters of the typical spelt, on the other hand, are quite different (fig. 1, A). The glume is stiff and thick, with a very blunt apex. It is strongly keeled and has no depression above the base, which is wide and firmly attached to the rachis.

Some wheats exhibit some of the spelt characters in a very weak form. For instance, some are more or less strongly keeled or have a flat shoulder at the distal part of the glume. While such wheats are classified as *T. sativum*, they are not true *sativum* in the sense used here. They do not represent the type.

Aside from the glumes, these species have other distinguishing characters. Of these, the brittleness of the rachis and nonshattering qualities of the spikelet in spelt may be mentioned as contrasted with the *sativum* types, the latter being nonbrittle and shattering easily.

Although the heads of the spelt varieties commonly grown at experiment stations in this country usually are lax, laxness of the head is not necessarily a characteristic of the spelt. Compactness may easily be introduced into spelts when crossed with *T. compactum*, which is really only a wheat carrying a genetic factor or factors for compactness.

The characters differentiating these two species are recapitulated below. This list includes also some other minor characters.

| *T. sativum.* | *T. spelta.* |
|---|---|
| Outer glumes— | Outer glumes— |
| Weakly attached by narrow base. | Firmly attached by wide base. |
| Weakly keeled. | Strongly keeled. |
| Apex tapering. | Apex blunt. |
| Depression or wrinkle near point of attachment. | No wrinkle near base. |
| Veins not prominent. | Prominent parallel longitudinal veins. |
| Shoulder narrow to broad, with no dentation. | Shoulder broad, with two or three dentations. |
| Glume soft. | Glume, lemma, and palea firm and thick. |
| Spikelets— | Spikelets— |
| Kernel loosely held between glumes (shattering). | Kernel tightly held (nonshattering). |
| Spikelets spreading somewhat from rachis. | Spikelets oppressed tightly against the rachis. |
| Usually three or more kernels per spikelet. | Usually two and rarely three kernels per spikelet. |
| Rachis— | Rachis— |
| Tenacious. | Fragile. |

There also are differences in the shape of the kernels of these two species (*1*).[1]

## LINKAGE OF SPECIFIC CHARACTERS

In order to understand the manner in which these two forms are here classified, it is necessary to refer to the linkage of their specific characters, without discussing the details.

Most of the characters tabulated above show two limits of contrasts. When a wheat is crossed with a spelt, a gradation of forms naturally

[1] Reference is made by number (italic) to "Literature cited," p. 363–364.

appears in the $F_2$ generation, ranging from the true spelt to the typical wheat form. In the segregates, a plant which has the glume form of the spelt invariably has a brittle rachis and a nonshattering habit. If the glume form is intermediate, the brittleness and shattering qualities also are intermediate. These three characters do not segregate independently. A plant with *sativum* glumes, for instance, has not been found whose rachis is brittle (a characteristic of the spelt only), nor have we obtained a spelt-glumed plant which has as tenacious a rachis as that of *T. sativum.* These species may present other characters, such as pubescence, beardedness, glume color, etc. These are common to both and segregate independently irrespective of the species. The presence of correlation between some of these specific characters in *sativum* × *spelta* crosses also has been noted by von Tschermak (*12*).

Unless the linkage is absolute it does not exclude the possibility of the occurrence of crossovers, but if crossing over ever occurs with respect to these characters it must be very rare.

The linkage of specific characters of spelt is very similar to the linkage of a number of glume characters of the wild oats in crosses between wild and cultivated forms described by Surface (*11*) and by Love and Craig (5).

It is absolutely necessary to bear in mind that the determination of the species is based only upon the presence, intensity of development, or absence of the specific characters (glume characters, brittleness of rachis, and seed-holding habit), all of which are linked to a very large extent. These characters show no independent segregation. It is obvious, therefore, that they do not mendelize independently. The characters which do segregate independently, such as pubescence, beardedness, glume color, etc., are not taken into consideration.[1]

## METHOD OF CLASSIFYING THE FORMS

The second and subsequent filial generations of *spelta* × *sativum* crosses show numerous gradations between the two parental forms. Some of the $F_2$ plants produced a progeny consisting of individuals which were decidedly spelts, while others produced a progeny which, while all spelts, yet resembled *T. sativum* to some extent and were distinctly different from the former group of plants where all the individuals were markedly spelt-like.

Ten arbitrary classes were made in order to record the degree of inheritance of spelt characters. The typical or intense spelt which exhibited all the specific spelt characters in their extreme forms was graded 1. As the exhibition of the intensity of these characters diminished—that is, as they tended to approach those of the wheat—the heads were

[1] Even these independently segregating characters vary in intensity and quality in these two species when segregating in crosses.

classed 2, 3, and so on to 9. Class 10 includes only true wheats, the forms which show absolutely no trace of the spelt characters. It must be emphasized that these 10 classes are purely arbitrary, based upon the appearances of the heads, and are not intended to represent any genotypic classes. The types representing these 10 classes are presented in Plate 33, A.

When the degree of inheritance of the spelt characters by a heterozygous population is recorded on two different occasions, a variation in the class frequencies naturally may be expected, but the general form of the curve remains practically the same. The error which may affect the conclusions rests in the decision as to whether a particular plant belongs to class 9 or 10. The degree of error depends upon the cross examined.

Of the plants discussed herein, which were tested in the $F_3$ generation, there were five cases where individuals belonging in class 9 had been placed in class 10 and only one case where a class 10 individual was erroneously recorded as belonging to class 9. In the tables these six corrections have been made. As the conclusions are based not merely on the $F_2$ individuals but upon the progeny of these, it does not seem likely that this source of error could have affected the results to any extent.

The spelt parents used for making the crosses graded from 1 to 4; the variety of wheat known as Gatineau [1] and herein considered as speltoid in form graded from 4 to 7; and all the *T. sativum* parents of course graded 10. As the discussions in this paper are confined only to the spelt character it does not seem necessary to describe further the agronomic and botanical characteristics of the plants used in making the crosses, as these have no direct bearing on the subject.

## FAMILIES SHOWING THE PRESENCE OF ONLY ONE FACTOR FOR SPELTING [2]

The hybrids of the first filial generation ($F_1$) of a *spelta* × *sativum* cross are slightly intermediate in form, resembling the spelt more than the *sativum* parent. They grade from 4 to 7, depending upon the cross. They possess all the characteristics of a spelt, but the spikelets may be somewhat more open and the grains may not be so firmly held within the glumes. The spelt characters are so nearly completely dominant that they inhibit all wheat characters.

In the second generation a segregation is obtained where the individuals vary, producing forms ranging from the typical wheat form to

[1] This variety originated as a cross of Red Fife ♀ (*T. sativum*) and Goose ♂ (*T. durum*) (*10, p. 239*). It is a striking illustration of the spelt like segregates which appear when these two wheat species are crossed. The heads are so much like spelt that at blooming time especially it is easily mistaken for spelt, but it thrashes free and is otherwise like the common wheats.

[2] For brevity, "spelting" is used throughout this paper in place of "inheritance of the spelt characters."

the spelt. The curve of these gradations is not a binomial frequency curve, but, on the contrary, more individuals are found, as a rule, at the extremities of the range than near the center. This fact may be observed in Table I, which shows the forms of the $F_2$ segregates.

TABLE I.—*Number of plants of the $F_2$ generation falling into each of 10 classes, based on presence or absence of spelt characters, with total number of spelts and wheats actually obtained, calculated numbers on basis of 3 to 1 ratio, deviation, probable error, and ratio between deviation and probable error*

| Series. | Crosses. | Number falling in class— | | | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 13255a | Spelt sel. 13440×Dale Gloria sel. 13401 | 17 | 10 | 9 | 5 | 4 | 1 | 3 | 1 | 2 | 21 | 73 |
| 13260a | Spelt sel. 13438×Turkey (C. I. 3375) sel. 13389 | 17 | 13 | 5 | 5 | 2 | 3 | 2 | ... | 2 | 14 | 63 |
| 13263a | Dale Gloria sel. 13401×spelt | 5 | 3 | 2 | 3 | 1 | ... | 2 | 2 | ... | 7 | 25 |
| 3004a | Black Bearded spelt×Early Red Chief | 19 | 7 | 5 | ... | 3 | 4 | 3 | 5 | 6 | 29 | 81 |
| 3085a | Black Bearded spelt×Jones Longberry | 9 | 8 | 4 | 2 | 4 | ... | 4 | 2 | 4 | 13 | 50 |
| 13124a | Vulgare (C. I. 3338)×spelt sel. 13437 | 9 | 8 | 4 | 0 | 7 | 4 | 3 | 5 | 7 | 20 | 76 |
| 13125a | Crimean (C. I. 3340) sel. 13351×spelt sel. 13437 | 6 | 3 | 9 | 3 | 4 | 2 | 5 | 5 | 4 | 9 | 50 |
| | Total of crosses | | | | | | | | | | ... | 418 |

| Series. | Crosses. | Number of plants. | | | | Deviation. | Probable error. | Ratio Dev./P. E. |
|---|---|---|---|---|---|---|---|---|
| | | Obtained. | | Calculated | | | | |
| | | Spelts. | Wheats. | Spelts. | Wheats. | | | |
| 13255a | Spelt sel. 13440×Dale Gloria sel. 13401 | 52 | 21 | 54.8 | 18.2 | 2.8 | 2.5 | 1.12 |
| 13260a | Spelt sel. 13438×Turkey (C. I. 3375) sel. 13389 | 49 | 14 | 47.3 | 15.7 | 1.7 | 2.3 | .74 |
| 13263a | Dale Gloria sel. 13401×spelt | 18 | 7 | 18.8 | 6.2 | .8 | 1.4 | .57 |
| 3049a | Black Bearded spelt×Early Red Chief | 52 | 29 | 60.8 | 20.3 | 8.8 | 2.6 | 3.38 |
| 3085a | Black Bearded spelt×Jones Longberry | 37 | 13 | 37.5 | 12.5 | .5 | 2.1 | .24 |
| 13124a | Vulgare (C. I. 3338)×spelt sel. 13437 | 56 | 20 | 57.0 | 19.0 | 1.0 | 2.5 | .40 |
| 13125a | Crimean (C. I. 3340) sel. 13351×spelt sel. 13437 | 41 | 9 | 37.5 | 12.5 | 3.5 | 2.1 | 1.67 |
| | Total of crosses | 305 | 113 | 313.5 | 104.5 | 8.5 | 6.0 | 1.42 |

As it is impossible to distinguish the homozygous spelts from the heterozygous forms, in determining the ratios all the spelt and speltoid forms (classes 1 to 9) have been grouped together and compared with

the wheat forms (class 10), which show no trace of spelt characters. The proportions between *spelta* and *sativum* forms of each of the crosses taken separately and that of the totals of these two groups approximated the monohybrid ratio of 3 to 1. The obtained ratio of the totals of the crosses was 305 speltoid and spelt forms to 113 wheats, the expections being 313.5 to 104.5, respectively, showing a deviation of 8.5 with a probable error of ± 6.0.

Two of the $F_2$ families, 13260a and 13255a, gave the results shown in Table II when the $F_3$ generation was grown.

Table II shows that the $F_2$ population of the families tested consisted of individuals in the proportion of 1 homozygous spelt to 2 heterozygous forms and 1 homozygous wheat.

TABLE II.—*Number of $F_2$ plants from series 13260a and 13255a which proved to be homozygous spelts, heterozygous forms, and homozygous wheats (1 : 2 : 1) when tested in the $F_3$ generation*

| Nature of data. | 13260a, Spelt × Turkey | | | | 13255a, Dale Gloria × Turkey. | | | |
|---|---|---|---|---|---|---|---|---|
| | Homozygous spelts. | Heterozygous forms. | Homozygous wheats. | Total. | Homozygous spelts. | Heterozygous forms. | Homozygous wheats. | Total. |
| Obtained | 8 | 13 | 9 | 30 | 7 | 14 | 12 | 33 |
| Calculated | 7.5 | 15.0 | 7.5 | ...... | 8.2 | 16.5 | 8.2 | ..... |
| Deviation | .5 | 2.0 | .5 | ...... | 1.2 | 2.5 | 3.8 | ...... |

The ratios of the totals of the forms produced in the $F_3$ generation by the heterozygous $F_2$ individuals of these two families (Table III) seem to conform to the foregoing assumption, although the ratios of the forms produced by each of the $F_2$ individuals sometimes are not so close to the 3 to 1 expectation. Of the total individuals produced by the $F_2$ heterozygous plants of series 13260a (spelt × Turkey), 212 were spelts and 71 wheats. These results were surprisingly close to the expectation, the deviation from the calculated numbers being but 0.3 with a probable error of ±4.9. In series 13255a (Dale Gloria × spelt), the numbers obtained from the heterozygous individuals were 365 spelts and speltoids and 156 wheats; the deviation here was 25.8 with a probable error of ±6.7. This apparent dominance of the spelt character over that of the wheat and its segregation into the 3 to 1 ratio are in accord with the observations of Pitsch, as cited by von Tschermak (*12, p. 179*) and of Kajanus (*3*).

TABLE III.—*Numbers of spelts and wheats produced in the $F_3$ generation from the $F_2$ heterozygous plants, and comparison of these with theoretical expectations, calculated on the 3 to 1 basis*

| Pedigree No. | Total $F_3$ plants. | Number of plants | | | | Deviation. | Probable error. | Ratio Dev./P. E. |
|---|---|---|---|---|---|---|---|---|
| | | Obtained. | | Calculated. | | | | |
| | | Spelts. | Wheats. | Spelts. | Wheats. | | | |
| 13260a-3 | 23 | 13 | 10 | 17.3 | 5.7 | 4.3 | 1.4 | 3.1 |
| 6 | 20 | 16 | 4 | 15.0 | 5.0 | 1.0 | 1.3 | .8 |
| 7 | 28 | 20 | 8 | 21.0 | 7.0 | 1.0 | 1.5 | .7 |
| 9 | 17 | 10 | 7 | 12.8 | 4.2 | 2.8 | 1.2 | 2.3 |
| 10 | 23 | 18 | 5 | 17.3 | 5.7 | .7 | 1.4 | .5 |
| 13 | 24 | 17 | 7 | 18.0 | 6.0 | 1.0 | 1.4 | .7 |
| 14 | 17 | 13 | 4 | 12.8 | 4.2 | .2 | 1.2 | .2 |
| 15 | 20 | 16 | 4 | 15.0 | 5.0 | 1.0 | 1.3 | .8 |
| 19 | 29 | 26 | 3 | 21.8 | 7.2 | 4.2 | 1.6 | 2.6 |
| 25 | 17 | 13 | 4 | 12.8 | 4.2 | .2 | 1.2 | .2 |
| 28 | 17 | 12 | 5 | 12.8 | 4.2 | .8 | 1.2 | .7 |
| 29 | 18 | 16 | 2 | 13.5 | 4.5 | 2.5 | 1.2 | 2.1 |
| 30 | 30 | 22 | 8 | 22.5 | 7.5 | .5 | 1.6 | .3 |
| Total | 283 | 212 | 71 | 212.3 | 70.7 | .3 | 4.9 | .1 |
| 13253a-7 | 36 | 34 | 2 | 27.0 | 9.0 | 7.0 | 1.7 | 4.1 |
| 8 | 19 | 15 | 4 | 14.3 | 4.7 | .7 | 1.3 | .5 |
| 10 | 24 | 20 | 4 | 18.0 | 6.0 | 2.0 | 1.4 | 1.4 |
| 11 | 35 | 27 | 8 | 26.3 | 8.7 | .7 | 1.7 | 4.1 |
| 15 | 59 | 44 | 15 | 44.3 | 14.7 | .3 | 2.2 | .1 |
| 16 | 33 | 23 | 10 | 24.8 | 8.2 | 1.8 | 1.7 | 1.1 |
| 17 | 13 | 4 | 9 | 9.8 | 3.2 | 5.8 | 1.0 | 5.8 |
| 21 | 52 | 28 | 24 | 39.0 | 13.0 | 11.0 | 2.1 | 5.2 |
| 23 | 49 | 29 | 20 | 36.8 | 12.2 | 7.8 | 2.0 | 3.9 |
| 24 | 35 | 18 | 17 | 26.3 | 8.7 | 8.3 | 1.7 | 4.9 |
| 25 | 44 | 33 | 11 | 33.0 | 11.0 | 0 | ..... | ..... |
| 26 | 37 | 25 | 12 | 27.8 | 9.2 | 2.8 | 1.8 | 1.6 |
| 28 | 50 | 33 | 17 | 37.5 | 12.5 | 4.5 | 2.1 | 2.1 |
| 30 | 35 | 32 | 3 | 26.3 | 8.7 | 5.7 | 1.7 | 3.4 |
| Total | 521 | 365 | 156 | 390.8 | 130.2 | 25.8 | 6.7 | 3.9 |

## FAMILIES SHOWING THE PRESENCE OF TWO SPELT FACTORS

Of the crosses studied, two families, 13126a (Giant Squarehead × spelt) and 3019a (spelt × Salt Lake Club) produced a very low proportion of wheat types. Not much importance would have been attached to the irregular behavior of these families if an apparently similar behavior had not been observed in another *sativum* × *spelta* cross. The manner of segregation of the progeny of these two crosses is given in Table IV.

TABLE IV.—*Degree of spelting and proportions of spelts and wheats obtained in the $F_2$ generations of spelta × sativum crosses which did not segregate in the 3 to 1 ratio*

| Series. | Degree of spelting in class— | | | | | | | | | | Total. | Number of plants. | | | | Deviation. | Probable error. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Obtained. | | Calculated. | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | Spelt. | Wheat. | Spelt. | Wheat. | | |
| 13126a [1] | 12 | 7 | 7 | 6 | 9 | 8 | 3 | 6 | 12 | 4 | 74 | 70 | 4 | 69.4 | 4.6 | 0.6 | 1.40 |
| 3019a [2] | 27 | 15 | 9 | 7 | 3 | 5 | 2 | 5 | 8 | 2 | 83 | 81 | 2 | 77.8 | 5.2 | 3.2 | 1.49 |

[1] Series 13126a, Giant Squarehead (C. I. No. 3351, selection 13366) × spelt (selection 13437).
[2] Series 3019a, white spelt × Salt Lake Club.

In series 13126a (Table IV), only 4 wheats were produced in a population of 74 $F_2$ plants, while in series 3019a, 2 wheats were produced in an $F_2$ population of 83 individuals. The deviation from the 3 to 1 ratio is so great that even by grouping class 9 with class 10—that is, by making generous allowances for observational error—the proportion approached more nearly the 15 to 1 ratio. On the basis of the 15 to 1 ratio, the expectation in series 13126a, is 69.4 to 4.6, in series 3019a, 77.8 to 5.2. The deviations are 0.6 and 3.2, and the probable errors $\pm 1.40$ and $\pm 1.49$, respectively.

On examining the $F_3$ generation produced from 27 plants of series 13126a, it was found that 12 of these had produced only spelts (Table V), two plants yielded only *sativum* types, and the remaining 13 $F_2$ plants yielded progeny of mixed forms. Assuming that the spelt parent in this particular cross carried two spelt factors, $S_1$ and $S_2$,[1] the first two generations will consist of the following genotypic forms:

| | | | | |
|---|---|---|---|---|
| $P_1$ | (Giant Squarehead) *T. sativum* | $s_1s_1s_2s_2 \times S_1S_1S_2S_2$ | | (Winter spelt) *T. spelta* |
| $F_1$ | | $S_1s_1S_2s_2$ (Speltoid) | | |
| $F_2$ | 1 $s_1s_1s_2s_2$ | 1 $S_1S_1S_2S_2$<br>2 $S_1S_1S_2s_2$<br>1 $S_1S_1s_2s_2$<br>2 $S_1s_1S_2S_2$<br>1 $s_1s_1S_2S_2$ | 4 $S_1s_1S_2s_2$ | 2 $S_1s_1s_2s_2$<br>2 $s_1s_1S_2s_2$ |
| Total | 1 *T. sativum*. | 15 spelts. | | |

If these genotypes were carried through the $F_3$ generation the theoretical behavior of each of the $F_2$ plants would be as follows

| GROUPS. | $F_2$ GENOTYPES | PHENOTYPES OF THE $F_2$ AND THEIR BEHAVIOR IN THE $F_3$ GENERATION. | |
|---|---|---|---|
| A | 1 $s_1s_1s_2s_2$ | 1 wheat will yield wheat only | |
| B | 1 $S_1S_1S_2S_2$<br>2 $S_1S_1S_2s_2$<br>1 $S_1S_1s_2s_2$<br>2 $S_1s_1S_2S_2$<br>1 $s_1s_1S_2S_2$ | 7 *spelts will yield spelts only.* | |
| C | 4 $S_1s_1S_2s_2$ | 4 spelts will segregate 15:1 | 8 unstable forms. |
| D | 2 $S_1s_1s_2s_2$<br>2 $s_1s_1S_2s_2$ | 4 *spelts will segregate 3:1* | |
| Total | | 16 | |

When the performances of the $F_2$ plants were examined, a close approximation was found to the above-mentioned theoretical ratios. The numbers of constant wheats, constant spelts, and unstable spelt forms obtained are shown in Table V, together with the theoretical expectations.

[1] In the factorial explanations given in this paper the spelt factors are assumed to be $S_1$ and $S_2$. Although the assumption of the factors $s_1$ and $s_2$ to stand for the wheat (*T. sativum*) character will fully agree with the results obtained, so far as the ratios go, there is no evidence as yet to warrant the assumption that the wheat and spelt characters are allelomorphic to each other. In fact, results with other specific crosses show the possibility that these are caused by two sets of independent factors. The behavior of the *sativum* × *spelta* crosses may be compared with the behavior of a maize cross where one parent has yellow endosperm and purple aleurone color (YYCCPP), and the other differs from this by its lack of purple color (YYCCpp). In such a cross, where the $F_2$ shows segregation into 3 purple to 1 yellow, the assumption that yellow and purple are allelomorphic may be used as a working hypothesis for crosses of this type, although it is not the correct explanation, as endosperm and aleurone color are two different characters altogether.

TABLE V.—*Frequencies of spelt and wheat classes in $F_3$ progeny of $F_2$ individuals of family 13126a, Giant Squarehead × spelt*

| Pedigree numbers of $F_2$. | Class of $F_2$ parent plant. | Classes of spelt inheritance $F_3$— | | | | | | | | | | Number of plants. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Spelts and speltoids. | Wheats. | Total. |
| FAMILIES CONSISTING OF WHEATS ONLY | | | | | | | | | | | | | | |
| 3 | 10 | | | | | | | | | | 4 | ... | 4 | 4 |
| 23 | 10 | | | | | | | | | | 10 | ... | 10 | 10 |
| FAMILIES CONSISTING OF SPELTS ONLY | | | | | | | | | | | | | | |
| 2 | 5 | 6 | 6 | 4 | 1 | 1 | | | | | | 18 | ... | 18 |
| 4 | 3 | 5 | 4 | 1 | | | | | | | | 10 | ... | 10 |
| 5 | 9 | | | | | | | | | 13 | | 13 | ... | 13 |
| 7 | 7 | | 2 | 6 | 1 | 2 | | 1 | 1 | | | 13 | ... | 13 |
| 8 | 3 | 9 | 2 | 1 | 1 | | | | | | | 13 | ... | 13 |
| 12 | 6 | | 1 | 2 | | | | | | 2 | | 5 | ... | 5 |
| 15 | 4 | 4 | 2 | 3 | 2 | 2 | 1 | | 1 | 3 | | 18 | ... | 18 |
| 16 | 5 | 3 | 1 | 3 | 1 | 2 | 1 | | 1 | 2 | | 14 | ... | 14 |
| 20 | 6 | 1 | | 1 | 1 | | | | | | | 5 | ... | 5 |
| 22 | ... | 2 | 3 | 2 | 3 | 1 | 1 | 1 | | 1 | | 14 | ... | 14 |
| 24 | 1 | 3 | 4 | 2 | | 1 | | | | | | 10 | ... | 10 |
| 25 | ... | | | | | | 1 | 3 | | 1 | | 5 | ... | 5 |
| Total | ... | | | | | | | | | | | 138 | ... | 138 |
| FAMILIES CONSISTING OF SPELTS AND WHEATS (HETEROZYGOUS $F_2$ PLANTS) | | | | | | | | | | | | | | |
| 1 | 5 | 1 | 2 | 5 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 16 | 1 | 17 |
| 6 | 6 | | 3 | 1 | 1 | 1 | | | 2 | 1 | 2 | 9 | 2 | 11 |
| 9 | 8 | | 2 | | | | | 1 | 1 | 1 | 2 | 5 | 2 | 7 |
| 10 | 9 | | | | | | | | | 3 | 1 | 3 | 1 | 4 |
| 11 | 8 | | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7 | 1 | 8 |
| 13 | 8 | | | | | | | | 1 | 13 | 1 | 14 | 1 | 15 |
| 14 | 7 | 2 | 3 | 1 | | 1 | | | | | 4 | 7 | 4 | 11 |
| 17 | 5 | 2 | 5 | 3 | 1 | | | | | 3 | 1 | 14 | 1 | 15 |
| 18 | 8 | 1 | 2 | 4 | 2 | 2 | 1 | | 1 | 3 | 1 | 16 | 1 | 17 |
| 19 | 1 | 1 | 3 | | | 1 | | | | | 1 | 5 | 1 | 6 |
| 21 | 1 | 2 | 5 | 5 | 2 | 1 | 1 | | | 2 | 4 | 18 | 4 | 22 |
| 26 | 9 | | | | | | | 1 | | 3 | 2 | 4 | 2 | 6 |
| 27 | 5 | | 1 | 1 | | 1 | | | 1 | 1 | 1 | 5 | 1 | 6 |
| Total progeny of heterozygous $F_2$ plants | ... | | | | | | | | | | | 123 | 22 | 145 |
| Expectations | | | | | | | | | | | | 108.75 | 36.25 | ...... |

SUMMARY AND GROUPING

| Groups. | Types. | Number of plants. | | Deviation. |
|---|---|---|---|---|
| | | Obtained. | Calculated 7:8:1. | |
| A | Wheats producing wheats only | 2 | 1.7 | 0.3 |
| B | Spelts producing only spelts | 12 | 11.8 | .2 |
| C and D | Spelts producing both spelts and wheats | 13 | 13.5 | .5 |

The agreement between the proportions expected and those obtained is very close indeed to the 7 to 8 to 1 ratio, and perhaps too close to be ordinarily expected from such a small population.

The analysis may be carried a step further. As shown above, the plants which would show unstability in the $F_3$ (groups C and D), were expected to be of two different genotypes. One of them, containing the $S_1s_1S_2s_2$ forms (group C), was expected to segregate in the 15 to 1 ratio, while the other (group D), containing the $S_1s_1s_2s_2$ and $s_1s_1S_2s_2$ genotypes, should segregate in the simple 3 to 1 monohybrid ratio. Apparently the individuals belonging to each of these two groups are those analyzed in Table VI.

Table VI.—*Analyses of unstable spelts of the $F_2$ generation series 13126a. Number of individuals of the $F_3$ generation produced from $F_2$ plants of groups C and D, compared with the theoretical expectation*

$F_2$ INDIVIDUALS APPARENTLY SEGREGATING IN THE 15 TO 1 RATIO (GROUP C, $S_1s_1S_2s_2$)

| Pedigree. | Total. | Number of plants: $F_3$ obtained: Spelts. | Number of plants: $F_3$ obtained: Wheats. | Number of plants: $F_3$ calculated: Spelts. | Number of plants: $F_3$ calculated: Wheats. | Deviation. | Probable error. | Ratio Dev./P. E. |
|---|---|---|---|---|---|---|---|---|
| 13126a-1 | 17 | 16 | 1 | 15.9 | 1.1 | 0.1 | 0.68 | 0.1 |
| 11 | 8 | 7 | 1 | 7.5 | .5 | .5 | .46 | 1.1 |
| 13 | 15 | 14 | 1 | 14.1 | .9 | .1 | .62 | .1 |
| 17 | 15 | 14 | 1 | 14.1 | .9 | .1 | .62 | .1 |
| 18 | 17 | 16 | 1 | 15.9 | 1.1 | .1 | .68 | .1 |
| Total | 72 | 67 | 5 | 67.5 | 4.5 | .5 | 1.38 | .3 |

$F_2$ INDIVIDUALS APPARENTLY SEGREGATING IN THE 3 TO 1 RATIO (GROUP D, $S_1s_1s_2s_2$ AND $s_1s_1S_2s_2$)

| Pedigree. | Total. | $F_3$ obtained: Spelts. | $F_3$ obtained: Wheats. | $F_3$ calculated: Spelts. | $F_3$ calculated: Wheats. | Deviation. | Probable error. | Ratio Dev./P. E. |
|---|---|---|---|---|---|---|---|---|
| 13126a-6 | 11 | 9 | 2 | 8.3 | 2.7 | 0.7 | 0.96 | 0.7 |
| 9 | 7 | 5 | 2 | 5.3 | 1.7 | .3 | .77 | .4 |
| 10 | 4 | 3 | 1 | 3.0 | 1.0 | 0 | ........ | ........ |
| 14 | 11 | 7 | 4 | 8.3 | 2.7 | 1.3 | .96 | 1.4 |
| 19 | 6 | 5 | 1 | 4.5 | 1.5 | .5 | .72 | .7 |
| 21 | 22 | 18 | 4 | 16.5 | 5.5 | 1.5 | 1.37 | .1 |
| 26 | 6 | 4 | 2 | 4.5 | 1.5 | .5 | .72 | .7 |
| 27 | 6 | 5 | 1 | 4.5 | 1.5 | .5 | .72 | .7 |
| Total | 73 | 56 | 17 | 54.8 | 18.2 | 1.2 | 2.50 | .5 |

Total $F_2$ plants segregating in 15 to 1 ratio, obtained, 5.
Total $F_2$ plants segregating in 15 to 1 ratio, calculated, 6.7.
Deviation, 1.7.
Total $F_2$ plants segregating in 3 to 1 ratio, obtained, 8.
Total $F_2$ plants segregating in 3 to 1 ratio, calculated, 6.7.
Deviation, 1.3.

The data in Table VI show that forms were obtained in the $F_2$ some of which segregated in the 15 to 1 and others in the 3 to 1 ratio as expected. The agreement to the theoretical numbers of the progeny of each $F_2$ plant is as close as can be expected with such small numbers, even

though it is borne in mind that the values of the probable error are likely to be too high in data of this kind.

Summing up the types of groups C and D, the former yielded 67 spelts to 5 wheats, and the latter group yielded 56 spelts to 17 wheats, the deviation being 0.5 and 1.2 and the probable errors $\pm 1.38$ and $\pm 2.50$, respectively.

Of the 27 plants tested (Tables V and VI), 5 (group C) showed an approximation to the 15 to 1 ratio and 8 (group D) to the 3 to 1 ratio. The theoretical number of plants belonging to each of the two unstable groups was 6.7—that is, one-fourth of the total $F_2$ plants tested. Comparing the results obtained with those expected, it will be noted that there were in the $F_2$ generation two (1.7 actual) $S_1s_1S_2s_2$ individuals less and one (1.3 actual) $S_1s_1s_2s_2$ or $s_1s_1S_2s_2$ more than expected (Table VI).

In considering the 15 spelt to 1 wheat segregation, it should be borne in mind that if the progeny of a heterozygous $F_2$ plant is less than about 10 individuals, the chances are that the wheat form, which is expected to appear but once in a population of 16 individuals, will not be obtained. Such heterozygous plants producing only spelts and no wheats, on account of their small $F_3$ population, would be classified under group B. Had $F_2$ plants 13126a – 12, – 20, and – 25 produced more than 4 or 5 individuals, 1 or 2 of them might have produced a wheat form which would have placed them in group C. The experimental ratios then would almost coincide with the theoretical.

Considering the closeness of agreement even in the details of the analysis, with such small numbers, there seems to be no question that we are dealing here with two spelt factors and that the ratio observed is the ratio of 15 to 1.

In the absence of more experimental evidence, the simplest hypotheses were given to account for the 3 to 1 and 15 to 1 segregations. Notwithstanding the surprisingly close agreements between the experimental and theoretical ratios, however, the real explanation concerning the production of the spelt character is still a matter of speculation.

The same spelt parent plant was used in crosses 13124a, 13125a, and 13126a. The wheat parents were of different varieties. No satisfactory explanation can be offered as to why the same spelt parent should produce a 15 to 1 ratio in cross 13126a and a ratio of 3 to 1 in crosses 13124a and 13125a. Three possibilities, however, may be mentioned.

1. The spelt plant used in the above-mentioned three crosses might have been heterozygous for one of the spelt factors. Such a heterozygous $S_1s_1S_2S_2$ plant bears gametes producing 3 to 1 and 15 to 1 ratios in the $F_2$ generation when crossed with a double recessive ($s_1s_1s_2s_2$) wheat.

2. The spelt parent may be assumed to have carried two spelt factors $S_1$ and $S_2$ and some of the wheats might have carried an inhibiting factor I. If the wheat carried the I factor the ratio of spelt to nonspelt would be about 3 to 1 and if it did not the ratio would be 15 to 1.

3. The spelt might have carried a spelt factor $S_1$ and in addition another factor $S_2$, which would produce the spelt character if there were present its complementary factor C, which might have been supplied by the wheat parent. In this case if C were present the ratio would be about 8 spelts to 1 wheat; and if C were not present, it would be 3 to 1. The ratios to be expected on the basis of this last possibility, however, are not in accord with the experimental results.

The second explanation seems to be the most plausible of the three. The assumption that some wheats carry the factor for the inhibition of the spelt character is not a mere speculation but a fact, as will be seen later when the question of the production of synthetic spelts by crossing two wheats is taken up. As commercial strains of *T. sativum* are not purified with respect to inhibitors, there are undoubtedly some strains which contain individuals heterozygous for this factor. If such a plant is used, for instance, as the female parent and is crossed with a spelt carrying the factor for spelting, according to the hypothesis some of the seeds will produce $F_2$ progeny where in some instances the ratio will suggest a 15 to 1, and in others a 3 to 1 segregation. As will be seen later, other modifications of these ratios may also be expected to arise.

PROGENY OF STABILIZED SPELTOID × SATIVUM CROSSES

The speltoid form used in the crosses which will be considered now is commercially known as "Gatineau." The variety originated from a cross between *T. durum* and *T. sativum*. It grades usually from 4 to 7 in the classification used in this study for spike form and so resembles the commercial spelts in this respect. It does not have the brittle rachis of spelt, and the grain thrashes from the glumes more easily than the grain of spelt, being like some of the tight-glumed wheats. It is neither a typical *spelta* nor a typical *sativum*.

The $F_1$ plants of the crosses between Gatineau and *T. sativum* were almost like Gatineau. The $F_2$ generation consisted of forms which were intermediate; typical spelts of classes 1 to 2 were not found. The classification of the $F_2$ individuals is reproduced in Table VII.

TABLE VII.—*Classes of $F_2$ generation plants of two speltoid × wheat crosses. Numbers of individuals obtained compared with the theoretical expectations*

| Series. | Degree of spelting. | | | | | | | | | | | Number of plants. | | | | Deviation. | Probable error. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Obtained. | | Calculated. | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total. | Speltoid. | Wheat | Speltoid. | Wheat | | |
| 13228a [1].... | 0 | 0 | 2 | 2 | 4 | 9 | 10 | 11 | 15 | 15 | 68 | 53 | 15 | 51.0 | 17.0 | 2.0 | 2.4 |
| 13229a [2].... | 0 | 0 | 1 | 0 | 5 | 9 | 7 | 9 | 15 | 17 | 63 | 46 | 17 | 47.2 | 15.8 | 1.2 | 2.3 |

[1] Series 13228a Turkey (C. I. 3375, Sel. 13389) × Gatineau (C. I. 2959, Sel. 13403).
[2] Series 13229a Seneca Chief (C. I. 3372, Sel. 13388) × Gatineau (C. I. 2959, Sel. 13403).

So far as the ratios of speltoid to wheat forms are concerned, these crosses segregated in the simple mendelian fashion. In series 13228a (Turkey × Gatineau) there were 53 speltoids and 15 wheats, showing a deviation from the theoretical numbers of 2.0 with a probable error of ±2.4 In series 13229a (Seneca Chief × Gatineau) there were 46 speltoids and 17 wheats, the deviation here being 1.2 with a probable error of ±2.3. In both cases the approximations of the figures obtained to those expected are within the range of their probable erros. Therefore, it can safely be concluded that the ratio is 3 to 1 and that there very likely is but one spelt factor difference.

The question of interest in the inheritance of this speltoid form (Gatineau) is not so much in its 3 to 1 ratio as in the way it differs in details from the spelt × wheat crosses first discussed, which segregated in the ratio of 3 to 1.

In order to compare the $F_2$ curves of these two groups of spelt × wheat crosses, the $F_2$ frequencies in Table VII (series 13228a and 13229a) and the first two series in Table I (13255a and 13260a) are represented graphically (fig. 2).

The comparative characteristics of the curves of these two sets of crosses are as follows:

The curves of the true spelt × wheat crosses (13255a and 13260a) begin at class 1, where they have their highest spelt frequencies. They gradually drop until they reach classes 5 to 9, inclusive, where there seems to be an indefinite fluctuation of frequencies. Then the curves suddenly rise again at class 10, which contains the spelt-free populations.

As to the curves of the wheat × speltoid crosses, the $F_2$ spelt populations begin at about class 3, where but a very few individuals are found. Beginning at class 5, the curves steadily rise until they reach their maximum height at class 10. The curves produced by the true spelt crosses, it will be recalled, continuously dropped instead of ascending.

When these two sets of crosses are compared it will be observed that, although there is but one spelt factor difference in each, the spelt factor present in crosses 13255a and 13260a is entirely different from the spelt factor present in crosses 13228a and 13229a. The wheat parent has had no influence is producing this variation in distribution, the same wheat parent plant, Turkey, C. I. No. 3375, selection 13389, having been used in crosses 13260a and 13228a.

## GENERAL DISCUSSION OF THE GROUPINGS WITHIN THE SPELT AND SPELTOID CLASSES

Having discussed the question of ratios, let us turn our attention to the analyses of the details of the variations within the spelt and speltoid classes.

The possibility of distinguishing the homozygous from the heterozygous spelts of the $F_2$ generation is of primary interest. Table VIII has

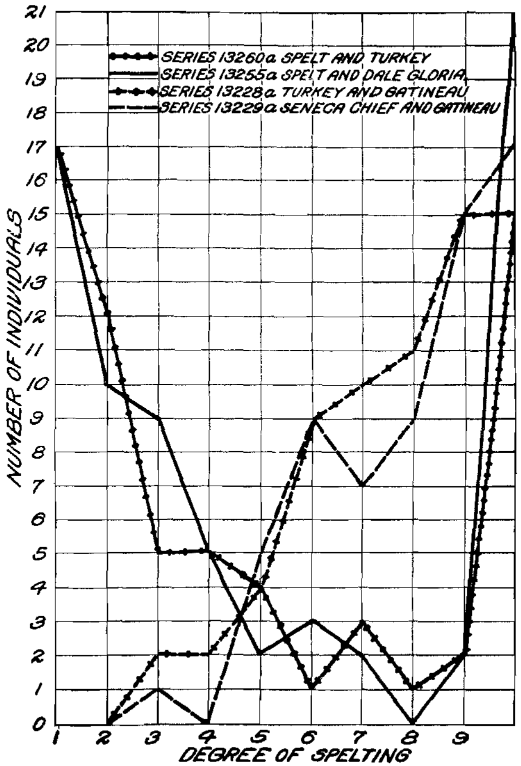


FIG. 2.—Degrees of spelting of $F_2$ population true spelt × sativum crosses (13255a and 13260a) as compared with the curves of sativum × speltoid crosses (13228a and 13229a) of the same generation.

been prepared to show these differences. Two sets of frequencies are represented. One set represents the classes of spelt inheritance of the $F_2$ plants of series 13255a and 13260a, which produced nothing but spelt in the $F_3$ generation. The other set shows the plants of the same generation and series which proved to be heterozygous for the spelt character and produced spelts as well as wheats in ratios approximating 3 to 1. These figures are taken from the data in Tables IX and X.

TABLE VIII.—*Comparison of classes of spelt inheritance of tested homozygous and heterozygous plants of the $F_2$ generation*

| Series. | Grades of spelting. | | | | | | | | | Totals. | Means. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | | |
| Homozygous spelts | | | | | | | | | | | |
| 13255a | 3 | 3 | 1 | | | | | | | 7 | 1.71 |
| 13260a | 1 | 5 | 1 | 1 | | | | | | 8 | 2.25 |
| Average of means | | | | | | | | | | | 1.98 |
| Heterozygous spelts. | | | | | | | | | | | |
| 13255a | 1 | 2 | 2 | 2 | 2 | 1 | 3 | | 1 | 14 | 4.64 |
| 13260a | 4 | 3 | 1 | | 2 | | 1 | | 2 | 13 | 3.69 |
| Average of means | | | | | | | | | | | 4.16 |

From Table VIII it is seen that the plants which proved to be homozygous for the spelt character occur from class 1 to class 4, the average of their means being 1.98. The $F_2$ plants which proved to be heterozygous, on the other hand, came from practically all classes, the average of the mean classes being 4.16.

Table VIII shows that, although there is no sharp difference in the phenotypic appearances of the homozygous and heterozygous individuals, yet as a rule the $F_2$ spelts of the speltoid classes (classes 5 to 9) are far more likely to be heterozygous for the spelt character than those of the true spelt classes (classes 1 to 4).

The comparison of the distributions of the $F_3$ populations of the $F_2$ homozygous spelts of the 3 to 1 and 15 to 1 segregating families is of special interest, as, among other things, it supports the two-spelt-factor explanations already given. The distributions of the $F_3$ populations of constant spelt-producing $F_2$ individuals of each of the three series 13126a, 13255a, and 13260a are recorded in Tables V, IX, and X, respectively, and are represented graphically in figure 3.

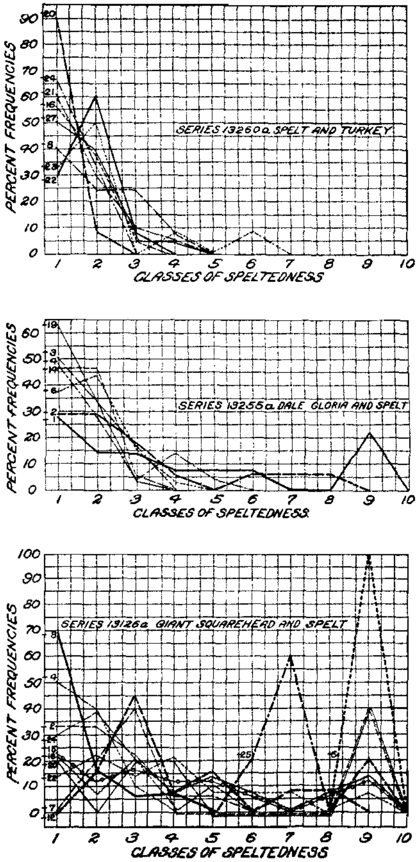


FIG. 3.—Comparison of $F_3$ generation curves of the progeny of $F_2$ spelts which yielded only spelts (series 13260a, 13255a, and 13126a).

TABLE IX.—*Frequencies of spelt and wheat classes in $F_3$ progeny of $F_2$ individuals in family 13255a, Dale Gloria × spelt*

FAMILIES CONSISTING OF SPELTS ONLY

| Pedigree number $F_2$ | Class of $F_2$ parent plant. | Classes of spelt inheritance. 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Number of spelts and speltoids. | Number of wheats. | Totals. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 4 | 2 | 2 | 1 | 1 | 1 | ... | ... | 3 | ... | 14 | ..... | 14 |
| 2 | 2 | 5 | 5 | 3 | 1 | .. | 1 | 1 | 1 | ... | .... | 17 | .... | 17 |
| 3 | 1 | 3 | 2 | 1 | .. | ... | ... | .. | ... | | . | 6 | ... | 6 |
| 4 | 1 | 10 | 6 | 1 | 3 | 1 | .. | .... | .... | .... | .. | 21 | ..... | 21 |
| 6.. | 2 | 16 | 19 | 7 | 1 | ... | . | .... | .... | .... | . | 43 | .... | 43 |
| 14 | 1 | 7 | 7 | 1 | ... | . | | .... | .... | ... | .... | 15 | ..... | 15 |
| 19 | 3 | 16 | 8 | 1 | .... | .. | .. | .. . | .... | .. | .. | 25 | .... | 25 |

FAMILIES CONSISTING OF WHEATS ONLY

| Pedigree number $F_2$ | Class of $F_2$ parent plant. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Number of spelts and speltoids. | Number of wheats. | Totals. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5...... | 10 | | | | | | | | | | | 11 | 11 | 11 |
| 9 . | 10 | | | | | | | | | | | 25 | 25 | 25 |
| 12 .. | 10 | | | | | | | | | | | 13 | 13 | 13 |
| 13 . . | 10 | | | | | | | | | | | 17 | 17 | 17 |
| 18 .. . | 10 | | | | | | | | | | | 21 | 21 | 21 |
| 20 . | 10 | | | | | | | | | | | 19 | 19 | 19 |
| 22. . | 10 | | | | | | | | | | | 38 | 38 | 38 |
| 27. | 10 | | | | | | | | | | | 23 | 23 | 23 |
| 29 . | 10 | | | | | | | | | | | 29 | 29 | 29 |
| 31. | 10 | | | | | | | | | | | 20 | 20 | 20 |
| 32. . . | 10 | | | | | | | | | | | 33 | 33 | 33 |
| 33 .. | 10 | | | | | | | | | | | 14 | 14 | 14 |

FAMILIES CONSISTING OF WHEATS AND SPELTS

| Pedigree number $F_2$ | Class of $F_2$ parent plant. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Number of spelts and speltoids. | Number of wheats. | Totals. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7....... | 3 | 20 | 9 | 2 | ... | ... | ... | ... | .. | 3 | 2 | 34 | 2 | 36 |
| 8..... . | 3 | ... | ... | 1 | 3 | 4 | 3 | 2 | 2 | . | 4 | 15 | 4 | 19 |
| 10.... . | 2 | 2 | 5 | 2 | 3 | 2 | .. | 2 | 2 | 2 | 4 | 20 | 4 | 24 |
| 11. ... | 1 | 17 | 8 | 1 | 1 | ... | .... | ... | ... | ... | 8 | 27 | 8 | 35 |
| 15 . .. | 5 | 5 | 8 | 7 | 5 | 6 | 4 | 2 | 4 | 3 | 15 | 44 | 15 | 59 |
| 16. .. | 4 | 8 | 5 | 5 | 1 | 3 | .. | 1 | ... | .. | 10 | 23 | 10 | 33 |
| 17 . | 7 | ... | 2 | 1 | 1 | ... | .... | .... | .... | .... | 9 | 4 | 9 | 13 |
| 21 | 5 | 5 | 4 | 5 | 2 | 1 | 1 | 2 | 2 | 6 | 24 | 28 | 24 | 52 |
| 23. . .. | 7 | 9 | 3 | 6 | .... | 4 | .... | 2 | 2 | 3 | 20 | 29 | 20 | 49 |
| 24...... | 2 | 5 | 1 | .... | .... | 2 | .... | .... | 1 | .. | 17 | 9 | 17 | 26 |
| 25. . .. | 9 | .. | 1 | 4 | 4 | .... | .... | .... | ... | 24 | 11 | 33 | 11 | 44 |
| 26.. . . | 7 | | 2 | 2 | 2 | 2 | 2 | 1 | 3 | 11 | 12 | 25 | 12 | 37 |
| 28...... | 4 | 10 | 13 | 5 | 1 | ... | 2 | 2 | ... | .... | 17 | 33 | 17 | 50 |
| 30...... | 6 | 1 | 6 | 2 | 2 | .... | .... | .... | .... | 21 | 3 | 32 | 3 | 35 |
| Total progeny of heterozygous $F_2$ plants | | | | | | | | | | | | 356 | 156 | 512 |
| Expectations | | | | | | | | | | | | 384 | 128 | ..... |
| Probable error | | | | | | | | | | | | ±6.61 | ..... | ..... |
| Deviation | | | | | | | | | | | | 18 | ..... | ..... |
| Ratio between deviation and probable error | | | | | | | | | | | | 2.72 | ..... | ..... |

TABLE X.—*Frequencies of spelt and wheat classes in $F_3$ progeny of $F_2$ individuals of series 13260a, spelt × Turkey*

FAMILIES CONSISTING OF SPELTS ONLY

| Pedigree number $F_2$. | Class of $F_2$ parent plant. | Classes of spelt inheritance ($F_3$). 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Number of spelts and speltoids. | Number of wheats. | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | 2 | 5 | 3 | 3 | 1 | | | | | | | 12 | ... | 12 |
| 20 | 2 | 10 | 1 | | | | | | | | | 11 | ... | 11 |
| 21 | 4 | 12 | 5 | 2 | 1 | | | | | | | 20 | ... | 20 |
| 22 | 1 | 4 | 8 | 1 | | | | | | | | 13 | ... | 13 |
| 23 | 2 | 4 | 6 | | 1 | | 1 | | | | | 12 | ... | 12 |
| 24 | 2 | 2 | 1 | | | | | | | | | 3 | ... | 3 |
| 27 | 3 | 10 | 8 | 1 | 1 | | | | | | | 20 | ... | 20 |
| 16 | 3 | 9 | 6 | 1 | | | | | | | | 16 | ... | 16 |

FAMILIES CONSISTING OF WHEATS ONLY

| Pedigree number $F_2$. | Class of $F_2$ parent plant. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Number of spelts and speltoids. | Number of wheats. | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 10 | | | | | | | | | | 17 | ... | 17 | 17 |
| 2 | 10 | | | | | | | | | | 14 | ... | 14 | 14 |
| 4 | 10 | | | | | | | | | | 15 | ... | 15 | 15 |
| 5 | 10 | | | | | | | | | | 15 | ... | 15 | 15 |
| 11 | 10 | | | | | | | | | | 30 | ... | 30 | 30 |
| 12 | 10 | | | | | | | | | | 20 | ... | 20 | 20 |
| 17 | 10 | | | | | | | | | | 30 | ... | 30 | 30 |
| 18 | 10 | | | | | | | | | | 12 | ... | 12 | 12 |
| 26 | 10 | | | | | | | | | | 24 | ... | 24 | 24 |

FAMILIES CONSISTING OF SPELTS AND WHEATS

| Pedigree number $F_2$. | Class of $F_2$ parent plant. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Number of spelts and speltoids. | Number of wheats. | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 9 | | | 1 | | | | 1 | 5 | 6 | 10 | 13 | 10 | 23 |
| 6 | 1 | 11 | 5 | | | | | | | | 4 | 16 | 4 | 20 |
| 7 | 1 | 13 | 6 | 1 | | | | | | | 8 | 20 | 8 | 28 |
| 9 | 3 | 5 | 3 | 1 | 1 | | | | | | 7 | 10 | 7 | 17 |
| 10 | 1 | 10 | 5 | 3 | | | | | | | 5 | 18 | 5 | 23 |
| 13 | 2 | 10 | 3 | | | 1 | | | | 3 | 7 | 17 | 7 | 24 |
| 14 | 7 | | | | | | | | 3 | 10 | 4 | 13 | 4 | 17 |
| 15 | 5 | 2 | | 2 | 3 | 4 | 1 | 1 | 1 | 2 | 4 | 16 | 4 | 20 |
| 19 | 2 | 9 | 11 | 6 | | | | | | | 3 | 26 | 3 | 29 |
| 25 | 2 | 6 | 6 | 1 | | | | | | | 4 | 13 | 4 | 17 |
| 28 | 5 | 1 | 1 | 2 | | 2 | 3 | 1 | 1 | 1 | 5 | 12 | 5 | 17 |
| 29 | 1 | 6 | 5 | 3 | 1 | | | | 1 | | 2 | 16 | 2 | 18 |
| 30 | 9 | | | | 1 | | 1 | 2 | 8 | 10 | 8 | 22 | 8 | 30 |
| Total progeny of heterozygous $F_2$ plants. | | 73 | 45 | 20 | 6 | 7 | 5 | 5 | 19 | 32 | 71 | 212 | 71 | 283 |
| Expectations | | | | | | | | | | | | 212.25 | 70.75 | ... |
| Probable error | | | | | | | | | | | | ±4.91 | ... | ... |
| Deviation | | | | | | | | | | | | .25 | ... | ... |
| Ratio of deviation to probable error | | | | | | | | | | | | .05 | ... | ... |

The $F_3$ populations of the series segregating in the 3 to 1 ratio (13255a and 13260a) have a general tendency to produce the maximum frequencies at class 1, sometimes at class 2. In only 4 families out of 15 are there *individuals in classes above the fourth, 10 individuals in 248 receiving the*

higher classification. The few individuals found in these speltoid classes at present may be regarded as exceptions. Their significance will be considered later.

The distributions of the families in the series segregating in the ratio of 15 to 1 (13126a) (Table V) are entirely different. They do not take the general course described above. Some of them have very low frequencies at classes 1 and 2. Families 5, 7, and 12 have no individual in class 1, the population of family 5 being composed of class 9 individuals only. Family 25 produced its spelts in classes 6 to 9, inclusive. Of the 12 families being considered 6 produced class 9 individuals, while among the families segregating in the 3 to 1 ratio there is but one instance (13255a, family 1) where class 9 individuals have been produced.

The explanation of the increased variability of the constant spelt-producing families of series 13126a, as compared with series 13255a and 13260a, will be found in the factorial explanations given for these two groups of crosses.

Families 13255a and 13260a segregated in the simple monohybrid 3 to 1 ratio. By hypothesis, all the spelts producing only spelts are supposed to have the genotypic composition SS.

As to the cross 13126a which segregated in the 15 to 1 ratio, it was shown that there were five constant spelt forms, namely:

$$S_1S_1S_2S_2 \quad S_1S_1S_2s_2 \quad S_1S_1s_2s_2 \quad S_1s_1S_2S_2 \quad s_1s_1S_2S_2$$

Although these forms would keep on producing only spelts, they are not genotypically identical.

The fact that in the families segregating in the 3 to 1 ratio there was only one genotypic spelt form and in the family segregating in the 15 to 1 ratio five such forms were present may account for the increased variability among the pure-breeding spelts of the latter cross.

The $F_3$ progenies can not be separated into the five theoretical genotypic groups just referred to because, among other things, there is positive evidence that modifiers also are concerned which have the tendency to shift the classes one way or the other. This phase of the subject will next be discussed.

## MODIFICATION OF THE DEGREE OF INHERITANCE OF SPELT CHARACTERS, DUE TO THE PRESENCE OF MODIFYING FACTORS

For the consideration of the subject of modifiers the analyses will be confined primarily to the spelt classes (1 to 9, inclusive) of the progeny of the heterozygous $F_2$ individuals of series 13260a, shown in Table X. This family has been chosen because it represents a simple mode of segregation. Whatever is said about modifiers for this family will be found to apply just as well to the other families.

It has been shown that only one spelt factor was concerned in the cross under consideration. All the $F_2$ heterozygous plants had the formula

Ss and produced spelts and wheats in the 3 to 1 ratio. If these $F_2$ heterozygous plants have all the same genotypic coustitution with regard to the spelt factor we would expect to see them produce similar $F_3$ spelt distributions. These distributions obtained experimentally are far from being uniform. For instance, plant 13260a-3 (Table X) produced individuals mainly of classes 8 to 9, and plant 13260a-6 produced its spelts in classes 1 and 2 only. The progeny of 13260a-15, on the other hand, showed no definite grouping, the curve spreading from one extreme to the other.

It may be argued that (1) these variations are insignificant; (2) they may be due to variations in soil and other external conditions; or (3) they are merely nonheritable fluctuations.

These arguments may be answered easily:

1. In the first place, let us take the first two frequencies (classes 1 to 9, inclusive), namely, those of 13260a-3 and 13260a-6 (Table X). The means are 8.00 ± 0.29 and 1.31 ± 0.08. The difference between the two means is 6.69 ± 0.30. This difference, it is seen, is very significant. Similar striking differences will be found when the means of the other distributions are compared.

2. With regard to variations due to soil conditions and external factors, it is only necessary to mention that these plants were grown on the same plot of the experimental field. The pedigree numbers following the family number represent the numbers of the rows in which the progeny of each of the $F_2$ plants was grown. For instance, the progenies of plants 13260a-28, 29, 30 (at bottom of Table X) were grown in three adjacent rows, yet 13260a-28 was composed of individuals contributing to nearly all classes of spelting, 13260a-29 produced practically all typical spelts, and the progeny of 13260a-30 were nearly all speltoid forms approaching the wheat type. So this second objection may also be dismissed.

3. As to the nonheritability of these variations, the objection may be settled by comparing the $F_2$ and $F_3$ generations in terms of the coefficient of heredity. If these variations are nonheritable fluctuations, there should be no correlation between the $F_2$ and $F_3$. Putting the statement in the affirmative, if there is a significant correlation in the degree of spelting of parent and offspring of the $F_2$ and $F_3$, then it is a direct and indisputable proof that these variations are transmitted to the following generations—that is, they are heritable. The accompanying correlation table (Table XI) has been prepared with a view of determining the validity of this last objection. The x-axis represents the mean classes of the $F_3$ and the y-axis represents the classes of the $F_2$ individuals which produced these $F_3$ forms. In this table are included all the progeny of the heterozygous forms of series 13255a and 13260a in order to have a sufficient number of individuals. The coefficient of heredity as calculated is 0.880 ± 0.029. As this coefficient is over 0.5 and over 10 times its

probable error it may be regarded as being very significant. As there is a significant correlation between these two generations, the variations under consideration are not fluctuations due to external conditions but are hereditary variations.

TABLE XI.—*Correlation table showing the classes of spelt inheritance of $F_2$ heterozygous plants, with the average degree of spelting of the $F_3$ progeny of each $F_2$ plant (series 13255a and 13260a)*

| Class of spelting of $F_2$ parent plants | Classes of spelt inheritance ($F_3$): 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | | | 4 | 1 | | | | | |
| 2 | | | 1 | 1 | 2 | | 1 | | |
| 3 | | | 1 | | 1 | 1 | | | |
| 4 | | | | | 2 | | | | |
| 5 | | | | | | 2 | 2 | | |
| 6 | | | | | | | 1 | | |
| 7 | | | | | | 1 | | 2 | 1 |
| 8 | | | | | | | | | |
| 9 | | | | | | | | 1 | 2 |

Coefficient of heredity = 0.880 ± 0.029

If there were no interference due to modifiers, the curve of the spelt $F_3$ progeny of the heterozygous $F_2$ individuals would follow the spelt curve of the $F_2$ generation, as both the spelts of the $F_2$ curve and those of the $F_3$ curves of heterozygous $F_2$ individuals consist of SS and Ss spelt plants in the proportion of 1 to 2, respectively.

An examination of the $F_3$ spelts of heterozygous individuals in Table X shows that the curves of the 13 families vary considerably from the curve of the $F_2$ generation (Table I, series 13260a), although the curve for the totals is much the same.

Again, if there were no genetic interference, all the $F_3$ progeny curves produced by heterozygous $F_2$ plants would be expected to follow more or less the same course. The experimental results exhibit wide differences, as the comparison of the classes of individuals 13260a-3, -6, -7, etc., will readily show.

As the presence of multiple factors is entirely out of question, it being proved in this case that the parents of this cross differ in only one factor for spelting, the following explanation may be given to account for these variations. One or more sets of modifiers furnished by the spelt, by the wheat, or by both parents seem to be present where each set of modifiers was in a homozygous dominant condition in one parent and in the alternative condition or absent in the other parent. These modifiers in the presence of the S factor tend to intensify the spelt character.

An example may be given to illustrate the effect which a modifier may produce in a spelt × wheat cross. The modifier which may cause dilution of spelting may be represented by the factor D and may be assumed to be carried by the wheat parent. (If this factor were contributed by the spelt parent, the latter would have been a dilute spelt, which was not the case in these crosses.) The wheat parent will then be represented by ssDD and the spelt parent by SSdd. The genotypic forms of the successive generations will be as follows:

| | | | | |
|---|---|---|---|---|
| $P_1$ | SSdd spelt. | × | | ssDD wheat. |
| | | SsDd | | |
| $F_1$ | | *semidilute spelt.* | | |
| $F_2$ | 1 SSdd.<br>2 Ssdd. | 2 SSDd.<br>4 SsDd. | 1 SSDD.<br>2 SsDD. | 1 ssdd.<br>2 ssDd.<br>1 ssDD. |
| | 3 typical spelts. | 6 semidilute spelts. | 3 dilute spelts. | 4 nonspelts. |
| | | 12 spelts | | 4 wheats. |

This represents a ratio of 3 spelts of different grades to 1 wheat.

If we assume that the nature of the modifier were to produce intensification of spelt inheritance in the presence of factor S, which in this case may have been carried either by the spelt or by the wheat, we will have, in the $F_2$ generation, 3 intense spelts, 6 semi-intense spelts, 3 normal spelts, and 4 wheats.

Some of these spelts (intense, normal, or dilute) will breed true to those conditions; others will produce some or all of these forms in different proportions as expected on the factorial hypothesis. If more than one set of modifiers are present the types and their proportions naturally become rather complex.

If, in the crosses 13255a and 13260a, a diluting modifier has been introduced, we would occasionally expect among the spelts (homozygous or heterozygous) some which are grouped in the dilute speltoid classes. The $F_3$ population of 13126a–5 and –25 (Table V); 13255a–26 (Table IX); 13260a–3, and –14 (Table X); and a number of others represent such cases. The progeny of 13260a–20, –6, –10 and others may represent spelts carrying some intensifying factor.

probable error it may be regarded as being very significant. As there is a significant correlation between these two generations, the variations under consideration are not fluctuations due to external conditions but are hereditary variations.

TABLE XI.—*Correlation table showing the classes of spelt inheritance of $F_2$ heterozygous plants, with the average degree of spelting of the $F_3$ progeny of each $F_2$ plant (series 13255a and 13260a)*

Classes of spelt inheritance ($F_3$)

| Class of spelting of $F_2$ parent plants | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | | | 4 | 1 | | | | | |
| 2 | | | 1 | 1 | 2 | | 1 | | |
| 3 | | | 1 | | 1 | 1 | | | |
| 4 | | | | | 2 | | | | |
| 5 | | | | | | 2 | 2 | | |
| 6 | | | | | | | 1 | | |
| 7 | | | | | | 1 | | 2 | 1 |
| 8 | | | | | | | | | |
| 9 | | | | | | | | 1 | 2 |

Coefficient of heredity = 0.880 ± 0.029

If there were no interference due to modifiers, the curve of the spelt $F_3$ progeny of the heterozygous $F_2$ individuals would follow the spelt curve of the $F_2$ generation, as both the spelts of the $F_2$ curve and those of the $F_3$ curves of heterozygous $F_2$ individuals consist of SS and Ss spelt plants in the proportion of 1 to 2, respectively.

An examination of the $F_3$ spelts of heterozygous individuals in Table X shows that the curves of the 13 families vary considerably from the curve of the $F_2$ generation (Table I, series 13260a), although the curve for the totals is much the same.

Again, if there were no genetic interference, all the $F_3$ progeny curves produced by heterozygous $F_2$ plants would be expected to follow more or less the same course. The experimental results exhibit wide differences, as the comparison of the classes of individuals 13260a–3, –6, –7, etc., will readily show.

As the presence of multiple factors is entirely out of question, it being proved in this case that the parents of this cross differ in only one factor for spelting, the following explanation may be given to account for these variations. One or more sets of modifiers furnished by the spelt, by the wheat, or by both parents seem to be present where each set of modifiers was in a homozygous dominant condition in one parent and in the alternative condition or absent in the other parent. These modifiers in the presence of the S factor tend to intensify the spelt character.

An example may be given to illustrate the effect which a modifier may produce in a spelt × wheat cross. The modifier which may cause dilution of spelting may be represented by the factor D and may be assumed to be carried by the wheat parent. (If this factor were contributed by the spelt parent, the latter would have been a dilute spelt, which was not the case in these crosses.) The wheat parent will then be represented by ssDD and the spelt parent by SSdd. The genotypic forms of the successive generations will be as follows:

| | | | | |
|---|---|---|---|---|
| $P_1$ | SSdd spelt. | × | | ssDD wheat. |
| $F_1$ | | SsDd semidilute spelt. | | |
| $F_2$ | 1 SSdd. | 2 SSDd. | 1 SSDD. | 1 ssdd. |
| | 2 Ssdd. | 4 SsDd. | 2 SsDD. | 2 ssDd. |
| | | | | 1 ssDD. |
| | 3 typical spelts. | 6 semidilute spelts. | 3 dilute spelts. | 4 nonspelts. |
| | | 12 spelts. | | 4 wheats. |

This represents a ratio of 3 spelts of different grades to 1 wheat.

If we assume that the nature of the modifier were to produce intensification of spelt inheritance in the presence of factor S, which in this case may have been carried either by the spelt or by the wheat, we will have, in the $F_2$ generation, 3 intense spelts, 6 semi-intense spelts, 3 normal spelts, and 4 wheats.

Some of these spelts (intense, normal, or dilute) will breed true to those conditions; others will produce some or all of these forms in different proportions as expected on the factorial hypothesis. If more than one set of modifiers are present the types and their proportions naturally become rather complex.

If, in the crosses 13255a and 13260a, a diluting modifier has been introduced, we would occasionally expect among the spelts (homozygous or heterozygous) some which are grouped in the dilute speltoid classes. The $F_3$ population of 13126a–5 and –25 (Table V); 13255a–26 (Table IX); 13260a–3, and –14 (Table X); and a number of others represent such cases. The progeny of 13260a–20, –6, –10 and others may represent spelts carrying some intensifying factor.

In conclusion, it may be said with certainty that besides the S factor in series 13255a and 13260a and the $S_1$ and $S_2$ factors in 13126a, modifiers are present which tend to dilute or intensify the spelt character.

In statistical studies of density in wheat, the junior author has found two characters whose mode of inheritance is almost identical with that of the spelt character. When a dense wheat (*T. compactum*) is crossed with a lax wheat (*T. vulgare*) a 3 to 1 segregation is found in the $F_2$ generation. The $F_2$ density curve consists of two distinct curves. One of these is a skew curve in the dense classes which contains 75 per cent of the individuals. After a gap, the other curve, which is composed of the lax segregates containing the remaining 25 per cent of the $F_2$ population, begins. Although the $F_3$ progeny of these heterozygous dense plants of the $F_2$ generation invariably produce bimodal curves similar to that of the $F_2$ just described, their modes or the means of the dense and lax curves shift at times considerably toward the lax classes and sometimes toward the denser classes, much in the same manner as does spelting. A similar phenomenon has been observed by Nilsson-Ehle (*8*) among his dense×lax wheat crosses.

The other parallel case is density of the type just mentioned, but in this case the modifier is known to be the spelt factor itself. The curves of the progeny of the heterozygous dense individuals of dense wheat×spelt crosses have the general bimodal form, but the populations which, in addition to density, carry the S factor always have their density curves shifted toward the lax classes.

With some spelts, the S factor shifts the density curves so much toward the lax classes that this S has to be regarded also as an inhibiting factor for density.

Hull-lessness in oats, according to Love and McRostie (7), is inherited in a similar manner. While this character segregates in the simple mendelian ratio of 1 hulled to 2 intermediates to 1 hull-less, the intermediate forms vary appreciably as regards the percentage of hulled kernels they produce. By correlating the percentage of hull-lessness of parent and offspring, they have shown that these variations within the 1 to 2 to 1 ratio are hereditary.

The mode of inheritance of the spelt character as shown in Tables IX and X closely resembles also Castle's (*2*) case of hooded rats, which had for a time aroused considerable controversy for and against the question of inconstancy of unit characters. In numerous crosses between rats having the hooded pattern and the wild (totally pigmented) or the Irish (white belly) types the hooded pattern behaved as a mendelian recessive. The ratio of nonhooded to hooded $F_2$ offspring was 3 to 1, showing that the hooded condition is dependent upon a single factor difference. Among the hooded individuals a considerable degree of variation was observed with respect to the degree of the extension of this pattern. By making selections for many generations in plus and minus directions

Castle was able to increase and decrease the pigmented area. His belief then was that the variations observed in the race of hooded rats were not mere fluctuations but were hereditary variations in the sense that the factor for the hooded condition had undergone alterations.

The assumption of unit-factor inconstancy, which Castle applied to account for variation of pigmentation of his hooded rats of course can not be applied for the analogous variations in the groupings of the spelt individuals, for, if this were the case, variations in this same extent should have been present among the self-fertilized population from which the parental form was selected. The classes of spelt inheritance in the parental strain ranged from 1 to 4, the mode being between classes 1 and 2. No departures nearly as great as those found in the homozygous extracted spelts of the $F_3$ generation were observed among this parental population. The study of the $F_2$ generation shows clearly that either some modifier or modifiers were introduced by the nonspelt parent or were carried by the spelt parent, but these modifiers were reduced to a recessive state as a result of crossing.

## PRODUCTION OF SYNTHETIC WHEATS BY CROSSING TWO SPELTS, AND SYNTHETIC SPELTS BY CROSSING TWO WHEATS

The writers frequently have obtained synthetic spelts in interspecific crosses in wheat. No indication has been observed as to the possibilities of producing true wheats in crosses between two different nonwheat species. It is theoretically possible, however, that such forms eventually will be produced in crosses between certain kinds of spelts. This supposition may be explained by taking as an example the results of one of the experiments discussed at length in this paper.

In the case of cross 13126a it was shown in detail that two spelt factors $S_1$ and $S_2$ were involved; that the $F_2$ segregates which bred true to the spelt character were not all genetically identical; and that they were composed of five genotypic forms, namely:

$$S_1S_1S_2S_2 \quad S_1s_1S_2S_2 \quad S_1S_1S_2s_2 \quad S_1S_1s_2s_2 \quad s_1s_1S_2S_2.$$

As long as these forms are allowed to be selfed, as they are in nature, no wheats ever segregate; but, by hypothesis, in a number of crosses between these five forms, a certain proportion of wheats are expected to appear in the following manner:

1. Crosses producing no wheats:

$$S_1S_1S_2S_2 \times \text{any other genotype};\ S_1s_1S_2S_2 \times s_1s_1S_2S_2;\ S_1S_1S_2s_2 \times S_1S_1s_2s_2.$$

2. Cross where one out of every four $F_1$ plants will produce 6¼ per cent wheats:

$$S_1S_1S_2s_2 \times S_1s_1S_2s_2.$$

3. Crosses where half of the $F_1$ plants will produce 6¼ per cent wheats:

$$S_1S_1s_2s_2 \times S_1s_1S_2S_2;\ S_1S_1S_2s_2 \times s_1s_1S_2s_2.$$

4. Cross where all $F_1$ plants will produce 6¼ per cent wheats:

$$S_1S_1s_2s_2 \times s_1s_1S_2S_2.$$

If wheats segregate from these crosses it will prove further the correctness of the two-factor hypothesis. It will also lead to the expectation that genotypic forms similar to the above, and other combinations as well, exist among the so-called pure commercial spelt forms and when the proper cross is made among these commercial spelts, a certain number of synthetic wheats may be produced in the $F_2$ generation.

It is easy to understand how the wheat character, being distinctly hypostatic, may be carried from generation to generation by the spelt type. But how can the spelt type segregate from a wheat × wheat cross? How can one conceive the spelt factor, which is so pronouncedly epistatic to the wheat character, as being carried by a wheat without being manifested phenotypically? The explanation is simple. It was shown that modifiers are involved in these crosses. Common wheats occasionally carry modifiers which tend to dilute the spelt character. Some of these modifiers were shown to be so effective that they grouped all of the spelts in class 9. Most of the class 9 individuals, as recorded in the foregoing tables, resemble wheat so closely that no one would be likely to call them true spelts.

If a certain diluting modifier can shift the spelts to class 9, a group of these may readily shift the spelt to classes between 9 and 10. If these diluting factors are reduced to a homozygous dominant condition, the dilute spelt which will be classified as 10 will breed true to type and be considered as wheat, although from a genetic standpoint such a form is a spelt.

As long as such sorts are allowed to self-fertilize they will produce a so-called pure line consisting of a constant wheat type. Their spelt characteristics are exhibited only when crossed with a common wheat which carries the factor for dilution in a recessive state. In the second generation of this cross the segregates which carry the S factor with the factor for dilution in a recessive state; that is, SS dd, will be spelts.

Fortunately, experimental evidence can be cited to support this statement. One of the writers has observed at the Kansas Agricultural Experiment Station over 20 spelts among $F_2$ hybrid plants derived from a number of wheat × wheat crosses where one of the parents was a rust-resistant variety of winter wheat and the other was Preston, Marquis, or Haynes Bluestem, well-known varieties of spring wheats. These parental types and some of the spelt segregates are shown in Plate 33, B.[1]

None of the $F_1$ plants in these crosses were spelts, or at least passed for spelts, although they might have shown some spelt characteristics in a weak form. In the $F_2$ generation, however, depending upon the cross, the proportions of wheat to spelts varied roughly from 3 to 1 to over

[1] The authors are indebted to Professors John H. Parker and L. E. Melchers for allowing them to photograph these forms and use them in connection with this paper.

100 to 1, with some sets of crosses producing no spelt at all. Besides the true spelts, a number of speltoid forms also segregated.

The absence of the spelt type in the $F_1$ generation shows that the absence of the spelt character in one of the parents was due to the presence of an inhibitor in the parent plant which carried the S factor. If the appearance of the spelt form in the $F_2$ generation was due to complementary factors furnished by both parents, the spelt should have appeared in the $F_1$ generation. Such was not the case.

The cultures consisted of over a thousand $F_2$ plants. It is not now absolutely necessary to know how, or exactly in what proportion, these appeared. The purpose of citing these examples is to substantiate the views expressed above regarding the possibilities of producing synthetic spelts from wheat × wheat crosses, which might have been regarded as a mere speculation in the absence of this experimental evidence. The fact that there were no spelts grown near the $P_1$ or $F_1$ plants and that spelts appeared in more than one cross excludes the possibility of accidental or natural cross-fertilization.

These observations show that common wheats may carry the spelt factor, but the latter can not express itself because one or more diluting or inhibiting factors are carried with it. Some of these diluting factors may be regarded as inhibiting factors which are not totally dominant but produce intermediacy in a heterozygous state.

From the small percentage of spelts which appeared in the $F_2$ generation in some cases, it may be inferred that there is one and in some cases more diluting factors.[1]

The production of synthetic spelts in wheat × wheat crosses just considered is similar to the synthetic production of *T. dicoccum dicoccoides*, the so-called "Wild Wheat" of Palestine, in the *vulgare* × *durum* cross (*6*). The wild character, consisting of a number of interdependent specific characters, is strongly dominant over both the *sativum* and the *durum* types, as is the spelt type toward *sativum*. Yet in both instances the character showing strong dominance toward either of the parental forms was carried by one of the parents together with a factor inhibiting in one case the wild and in the other case the spelt characters.

## MODIFICATIONS OF MENDELIAN RATIOS

The question of modifiers whose presence in the production of the spelt character was demonstrated in various ways brings us to the consideration of the modifications of mendelian ratios. As all spelts or all wheats are not alike with respect to their ability to intensify, repress, or inhibit the production of the spelt characters, it is natural to expect

[1] Nilsson-Ehle (*8, 9*) and Kajanus (*3, 4*) have also observed the occurrence of spelts in certain *sativum* × *sativum* crosses. They support the correctness of the foregoing observations and tend to preclude the assumption of the possibility of the occurrence of some accidental or natural crossing of one of the *sativum* parents with a spelt.

certain modifications of the 3 to 1 ratio. For instance, in certain spelt × wheat crosses, depending upon the potency of the diluting factors, the proportion of wheats to spelts may increase in certain amounts. In some instances, the increase of wheats will be slight, so that the obtained deviation from the 3 to 1 ratio, which will be on the side of excess for the wheat class, will be considered within or near the limits of the probable error and the inheritance will be regarded as simple mendelian. Perhaps series 13255a, where an increased proportion of both constant breeding wheats and $F_3$ wheat segregates of heterozygous $F_2$ plants are obtained, represents such a case.

It also is possible that the ratio may fall between 3 to 1 and 1 to 1 in case the diluting factor is very strong. Here, then, will be an excess in the homozygous wheat class at the expense of the homozygous dominant spelt class. If factors of both dilution and of intensification are introduced in the same cross, the experimental ratios will defy any attempt at simple factorial explanations. The writers have obtained a cross where the $F_2$ generation suggested a possible 3 to 1 segregation, but on examining the $F_3$ generation, which was composed of a fairly large population, neither the individual segregations nor the totals of these approached in any way 3 to 1 or 1 to 2 to 1 expectations.

It naturally follows from the foregoing discussion that in spelt-wheat crosses wide departures from simple ratios occasionally may be expected.

## SUMMARY

*T. spelta* and *T. sativum* are differentiated by a number of linked specific characters, which are present in one species and absent in the other. These characters, so far as observed, are not inherited independently but are transmitted as a group.

In crosses between a spelt and common wheats the $F_1$ hybrid shows dominance of the spelt, but this character appears in a somewhat diluted form. In the second generation all classes of spelt inheritance are obtained. In order to classify these forms, 10 arbitrary classes were erected, class 1 representing the true spelt and class 10 the total absence of this character. The intermediate classes represent different grades of spelt inheritance.

In most of the material studied there was but one factor difference for spelt, but in two cases two spelt factors were present. Both 3 to 1 and 15 to 1 ratios were obtained. These ratios were verified after determining the genotypic constitution of the $F_2$ plants, which gave pure breeding spelts, inconstant spelts, and pure breeding wheats in the ratios of 1 to 2 to 1 and 7 to 8 to 1, respectively. Of the constant spelts produced in crosses with this latter ratio, approximately half yielded (in the $F_3$ generation) spelts and wheats in the ratio of 15 to 1 and the other half in the ratio of 3 to 1, as expected on the two factor hypothesis.

The speltoid form "Gatineau" when crossed with wheats gave also a 3 to 1 segregation of spelts and wheats, but the curves showing the classes of spelts produced by this cross were entirely different from the curves produced by the spelt-wheat crosses which segregated in the same ratio.

Aside from the factor or factors for spelting, there is positive evidence showing the presence of intensifying and diluting modifiers which tend to affect the degree of spelt characters without affecting to any extent the ratios of spelts to wheat. Some of the diluting modifiers tend to act as inhibitors.

The progeny of all heterozygous spelts of the Ss type do not produce a similar spelt curve. There are wide discrepancies in the spelt inheritance of the progeny of these forms. These variations within the spelt classes have been found to be hereditary and to be caused by modifiers.

The theoretical possibility of producing synthetic wheats in crosses between certain pure-breeding spelts is shown.

Experimental evidence also is presented showing that, in spite of the fact that the spelt character is dominant over the wheat form, the former may be synthetically produced by crossing certain wheats, provided one of the wheats carries a spelt factor together with an inhibitor and that the other wheat carries neither.

It is shown that, if intensifying, inhibiting, and diluting modifiers are introduced in a cross, wide departures may be expected from the 3 to 1 and 15 to 1 ratios.

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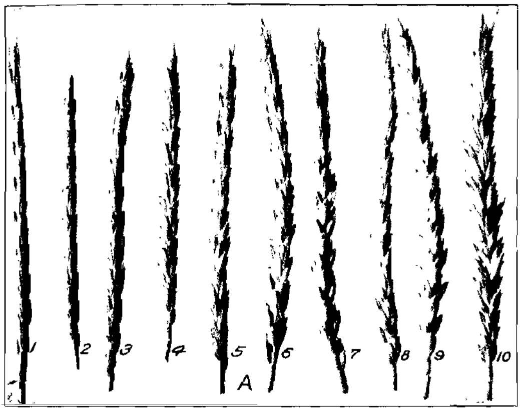
PLATE 33

**A.**—Wheat spikes showing different degrees of spelting. 1 and 2 are intense spelts; 3 to 9 are intermediate forms, showing varying degrees of dilution of the spelt character; 10 is a pure-breeding wheat (*sativum*) form, showing no trace of spelting. The numbers represent approximately the types falling in the 10 classes of spelting.

B.—Snythetic spelts produced in $F_2$ generation in wheat × wheat crosses; a, b, c, and d are the *sativum* parent plants; b–1 spelt form of the progeny of a × b; c–1 to c–5 spelt forms of the progeny of a × c; d–1 and d–2 spelt forms of the progeny a × d.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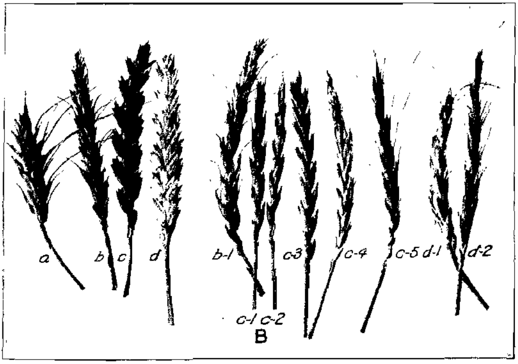
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c3
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d-1
d-2
B

# PLUM BLOTCH, A DISEASE OF THE JAPANESE PLUM, CAUSED BY PHYLLOSTICTA CONGESTA HEALD AND WOLF [1]

By JOHN W. ROBERTS, *Pathologist, Fruit Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

## INTRODUCTION

In June, 1905, W. M. Scott of the Bureau of Plant Industry, United States Department of Agriculture, collected near Fort Valley, Ga., fruits of the Japanese plum (*Prunus triflora* Roxbg.) affected with a disease very closely resembling the apple blotch, due to *Phyllosticta solitaria* E. and E. In the diseased areas were spore-bearing pycnidia which were found also on the leaves in gray papery spots resembling those on apple leaves caused by *Phyllosticta solitaria.* On May 27, 1908, the disease was again observed by Scott on both fruit and foliage of the Burbank plum at Montezuma, Ga. It was found to be rather common in several orchards about Montezuma, in some cases causing enough damage to injure seriously the market value of the fruit. In one orchard a large part of the fruit was affected, and many specimens bore from 15 to 20 spots each.

On May 29, 1917, the writer collected near the same locality Japanese plum fruits and leaves affected with the same disease. In the single orchard in which the disease was found, most of the fruit was heavily nfected and rendered nearly worthless. Considerable difficulty was encountered in finding the disease again, as the Japanese plum industry in Georgia had about passed out. Lack of demand for the fruit coupled with the susceptibility of all parts of the tree to various diseases and insect pests had caused growers either to eradicate their trees or to let them die. At present there are almost no Japanese plum orchards remaining in Georgia, and all of the trees in which plum blotch was found have been eradicated. So far as the writer knows, then, the disease no longer exists, though it is to be looked for throughout the South as far west as Texas. Should the growing of Japanese varieties of the plum be revived in the South, blotch may prove to be one of its most serious diseases, as it is very destructive, and probably would be exceedingly difficult to control.

The varieties found to be affected were Abundance, Burbank, and what was apparently an unnamed seedling.

[1] A brief description of this disease was published as an abstract of a paper presented at the Ninth Annual Meeting of the American Phytopathological Society. (ROBERTS, John W. PLUM BLOTCH. (Abstract.) *In* Phytopathology, v. 8, no. 2, p. 74. 1918.)

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## DESCRIPTION OF THE DISEASE

The infected parts on the unripe fruit appear as dark-colored raised areas with fringed margins and are somewhat roughened by the presence of small blisters and depressions (Pl. 34, B). As in the case of apple blotch, the skin often becomes ruptured as the fruit increases in size.

On the ripe fruit the blotched parts appear as irregular browned areas 3 to 6 mm. in diameter and consist of an aggregation of from 4 to 20 sunken spots, each separate spot being 1 mm. or less in diameter. At this stage the spots have a peculiar light blue cast owing to the "bloom" of the ripe plum covering the browned epidermis. The diseased area is rather superficial, extending only slightly below the epidermis. The affected tissues become hardened and somewhat leathery and show no tendency to decay.

Small, glistening pycnidia are produced in considerable numbers even in the younger spots. Quite commonly there are 25 to 30 scattered promiscuously about in each blotched area. Infection evidently takes place when the fruits are very young, since the spots found May 29 were well formed and bore pycnidia with mature spores. Judging from the writer's inoculation experiments, infection probably took place five to six weeks earlier, or about the middle of April.

On account of its characteristic appearance on the fruit, the disease has been given the common name of "plum blotch."

On the upper surface of the leaf blades (Pl. 34, A), the spots are angular, rather small (about 0.5 mm. across), brown when young, but later becoming gray or silvery in color. They may be numerous, as many as 200 sometimes appearing on a single leaf. Usually only a single pycnidium is present in each spot, except where two or more spots have coalesced to form a single large spot. Affected areas are also found on the petioles and on the veins of the lower surface, especially on the midrib. On these the diseased areas are much larger than on the upper surface of the blade and are black and sunken. Pycnidia, bearing spores, are present in great abundance.

Pycnidia, apparently identical with those found on the fruit and leaves, were found also in small light-colored, often slightly sunken areas on the twigs; but, as spores were lacking, positive identification could not be made. It is possible that these pycnidia had discharged their spores early in the spring and had brought about the early infections on the fruit.

## CAUSE OF PLUM BLOTCH

By comparison with type specimens, the organism involved in the production of plum blotch has been found to be identical with the fungus described by Heald and Wolf [1] as *Phyllosticta congesta*. Heald and

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[1] HEALD, F. D., and WOLF, F. A. NEW SPECIES OF TEXAS FUNGI. *In* Mycologia v. 3, no. 1, p. 8. 1911.

Wolf found the fungus on the leaves only of *Prunus* sp. in Texas Their description is as follows:

*Maculis minutis*, .5–.8 mm diam., brunneis numerosis, venis limitatis; pycnidiis solitariis in quaque area, 50–125 μ diam.; sporulis globulosis vel leniter elongatis, hyalinis 6–9 μ.

On Prunus sp. Boerne (Texas) 1554 (Type).

On the upper surface of the leaf are very numerous brown areolae bounded by the veins of the leaf. The lower surface may not be discolored. These minute spots fuse, and each contains at its center a single black pycnidium. The pycnidia contain globular or slightly oval, clear spores.

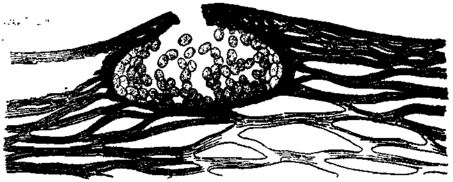


FIG. 1.—Section through a pycnidium of *Phyllosticta congesta*, showing spores. Natural infection on plum fruit, Georgia 1917. × 340.

Heald and Wolf do not mention the fact that the older spots become gray or silvery, though the type specimens as well as those collected by Scott and the writer show this to be the case. The spots on these leaves and those on Georgia specimens collected by the writer show a marked resemblance, and the fungi found upon them are morphologically the same. The spots on the leaves collected by the writer have a greater tendency to fall out.

The pycnidia (fig. 1) are glistening, lens-shaped, erumpent, on the leaves 65 to 120 μ in diameter, on the fruit 60 to 120 μ in diameter. On the average, pycnidia on the fruit are somewhat larger than those on the leaves. Spores on the leaves measured 7 to 9 μ in diameter, on the fruit 8 to 9 μ. Spores from younger spots were invested with gelatinous envelops which were sometimes lengthened into appendages (fig. 2). Spores from older spots do not show these envelops, and they are not to be found in the dried herbarium specimens. The young spores of *Phyllosticta solitaria* have such an envelop. In fact, *P. solitaria* and *P. congesta* resemble one another so closely that on purely morphological grounds they might be considered as identical. Since the ascogenous stage of neither fungus is known, the writer prefers to retain the name *P. congesta* as a matter of convenience, unless it is shown by cross inoculations that the fungus on the apple and that on the plum are identical in every way.

FIG. 2.—Spores of *Phyllosticta congesta*, with the gelatinous envelops which are sometimes present. From pycnidia on plum fruit, Georgia 1917. × 680.

Of course the final test of identity would lie in whether or not the ascogenous stages of the two fungi, assuming them to exist, would prove to be identical.

Specimens of *Phyllostica congesta* on fruit and foliage of *Prunus triflora* have been deposited in the Pathological Herbarium, Bureau of Plant Industry, United States Department of Agriculture.

It is not known how the fungus is carried over from one season to another. If it occurs on the twigs, as the writer is inclined to think, there would be good reason for believing that production of spores from twig lesions in the spring would constitute an important infection source. It is also possible that the fungus survives the winter on leaves and fruit.

On all the ordinary culture media the fungus shows about the same type of growth. On corn meal agar, beef agar, prune agar, potato agar, and oatmeal agar growth is very slow, and on all these media it presents the same appearance. There is a dense black mass of closely woven hyphae forming a raised and irregular aggregation of shining bead-like bodies which may be considered as sterile pycnidia, since they are more or less hollow bodies containing oil drops. The margin of the growth is often fringed; in fact on the above-named media the growth is almost as blotch-like as it is on the fruit of the plum. On sterilized stems of Melilotus the growth resembles that on the agars, but spores are often formed though very scantily.

On Japanese plum twigs growth is also very slow. Pycnidia and spores are formed in about two months. Pycnidia are formed on the bark and may also be formed at the cut end of the twig, in which case they are densely aggregated.

Sterilized apple twigs proved to be the best medium for the production of spores, though two to three months must elapse before spore production begins. On this medium the only sign of growth by the fungus is the formation of a dense mass of hyphae and pycnidia, closely aggregated at the upper end or at an abraided place on the side of the twig. On all the media used the type of growth exhibited by *Phyllosticta congesta* differs somewhat from that of *P. solitaria*. On sterile apple twigs, for instance, the latter produces pycnidia which are scattered over the bark, whereas the pycnidia of the former are formed only at the cut ends of the twigs.

In 1917 the fungus was isolated from both fruit and leaves by the poured plate method, using spores, and by planting bits of the diseased tissues in plates.

In the spring of 1918, no spores had been obtained in cultures, but inoculations were made by spraying the young fruits and leaves of Abundance and Burbank plums with bits of hyphae and sterile pycnidia suspended in sterile distilled water. The results were negative in every case.

In 1919, spores obtained from apple twig cultures and suspended in sterile distilled water were applied to fruit, foliage, and twig of Abundance plums on May 15. Where cultures originally obtained from plum fruits were used, two fruits were found with two typical blotches on each of them; three leaves were found with scattering spots, each spot typical of the disease and each bearing a single pycnidium with the characteristic spores of *Phyllosticta congesta*. Like results were obtained by the use of cultures obtained from the leaves; one fruit showed three typical blotches with pycnidia and two others showed one; seven leaves were successfully infected. From all these artificially inoculated parts, the fungus was reisolated and proved to be *P. congesta*.

No lesions were found on the twigs.

Inoculations made upon Japanese plums with spores from pure cultures of *Phyllosticta solitaria* gave negative results in 1918, 1919, and 1920, though the spores were applied to fruit, foliage, and twigs at frequent intervals throughout the spring.

Though the inoculation experiments herein reported upon are sufficient to prove *Phyllosticta congesta* the cause of plum blotch on leaves and fruit and show the fungus on the fruit to be identical with that on the leaves, they are not as complete as the writer should wish. All the inoculation work was done at Arlington, Va., under conditions probably unfavorable to the fungus, since it has been found naturally only in regions much farther south.

It is planned to carry on further inoculation work with both the plum blotch and apple blotch Phyllostictas. The writer expects eventually to obtain successful inoculations on plum twigs using *Phyllosticta congesta* as inoculum.

## CONTROL MEASURES

No attempts to control plum blotch have been made. One would expect that control might be had by spraying with a strong fungicide at intervals beginning shortly after the petals have been shed as is the case with apple blotch. Dilute lime-sulphur solution and Bordeaux mixture injure Japanese varieties of the plum so severely as to preclude their use during the growing season. It is also doubtful whether or not dilute lime-sulphur solution would control severe cases of disease, since it will control only mild cases of apple blotch. Self-boiled lime-sulphur can be used with safety on the Japanese plum, but it is a fungicide which is even weaker than dilute lime-sulphur solution. It seems probable, therefore, that should this disease ever become an important one, its control will present a problem of considerable difficulty, though it is realized that the reasoning by analogy in which the writer has just indulged may easily lead to wrong conclusions.

SUMMARY

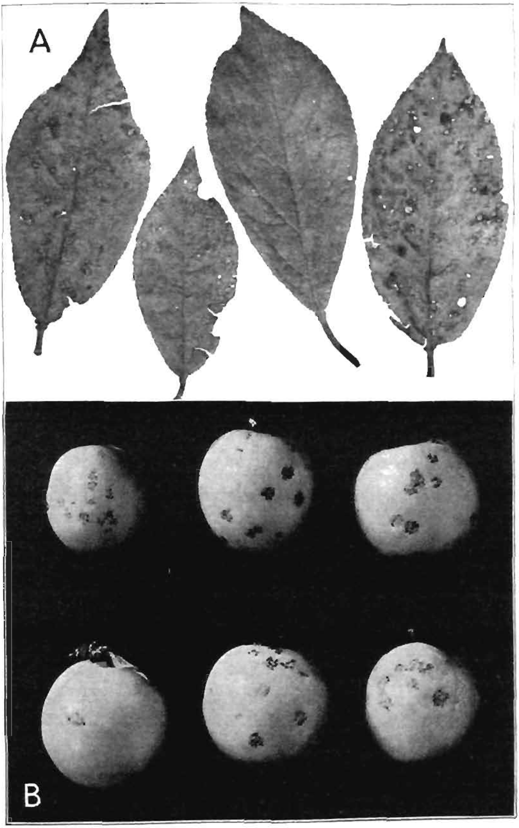
Plum blotch, a hitherto unknown disease of the Japanese plum (*Prunus triflora*), has been found in Georgia. In addition to the fruit, the leaves and possibly the twigs are affected. The lesions on fruit and leaves greatly resemble those of the apple caused by *Phyllosticta solitaria* E. and E.

Varieties Abundance and Burbank were found to be susceptible. An unnamed seedling, probably also belonging to *Prunus triflora* was found to be severely infected.

From diseased fruits and leaves the fungus, *Phyllosticta congesta* Heald and Wolf, was isolated and grown in pure culture. Spores obtained from cultures on sterile apple twigs when suspended in distilled water and sprayed on healthy fruits and leaves produced characteristic lesions of the disease.

*Phyllosticta congesta* Heald and Wolf is to be considered for the present as different from *P. solitaria* E. and E., though greatly resembling it. Inoculation experiments on plums using spores from pure cultures of *P. solitaria* were negative.

No attempts have been made to control plum blotch, but the possibilities of control are discussed.


A
B

# A COMPARISON OF THE PECTINASE PRODUCED BY DIFFERENT SPECIES OF RHIZOPUS

By L. L. Harter, *Pathologist*, and J. L. Weimer, *Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

## INTRODUCTION

Recent investigations by Harter and Weimer [1] showed that *Rhizopus tritici* Saito, an organism demonstrated to be parasitic on sweet potatoes, produces a powerful intracellular and extracellular pectinase which dissolves the middle lamella so that the cells readily separate without themselves undergoing any noticeable alteration. A suspension of 0.25 gm. of the enzym powder in 25 cc. of water was found to completely macerate sweet potato disks 1 mm. in thickness in two to five hours. Furthermore, the solution on which the fungus grew was even richer in pectinase, maceration of the sweet potato disks being completed in one to three hours.

Since the foregoing results have been published Harter, Weimer, and Lauritzen [2] have concluded experiments which showed that out of 11 species of Rhizopus studied 9 were parasitic on the sweet potato. Furthermore, these investigators found that the species differed in degree of parasitism, both as regards the percentage of infection and the rapidity of decay.

The present investigations, therefore, had for their object to determine (1) if pectinase is produced by all species of Rhizopus and, if so, to what extent and (2) if its production is any indication of the parasitism of the species.

## TECHNIC

The methods employed in carrying out macerating experiments with the different species of Rhizopus were for the most part the same as those used in previous work to which reference [1] has already been made, although some slight modifications were necessary to meet certain phases of the problem. Three sets of experiments were carried out with each organism. All the species were included in a single experiment and the macerating action was determined for all at the same time, so that the results for each species are directly comparable for a single experiment. The culture medium was so prepared and in sufficient quantity as to make

[1] Harter, L. L., and Weimer, J. L. STUDIES IN THE PHYSIOLOGY OF PARASITISM WITH SPECIAL REFERENCE TO THE SECRETION OF PECTINASE BY RHIZOPUS TRITICI SAITO. *In* Jour. Agr. Research, v. 21, no. 9, p. 609-625. 1921. Literature cited, p. 624-625.

[2] Harter, L. L., Weimer, J. L., and Lauritzen, J. I. THE SUSCEPTIBILITY OF THE DIFFERENT VARIETIES OF SWEET POTATOES TO DECAY BY RHIZOPUS NIGRICANS AND RHIZOPUS TRITICI. *In* Phytopathology, v. 11. 1921. In press.

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it uniform in all the flasks for all organisms. Four flasks (2 liters), containing 300 cc. of the culture medium, were inoculated with each one of the species in each experiment, and the cultures were incubated for three days.

The macerating action was determined for the following species of Rhizopus: *chinensis* Saito, *nodosus* Namysl, *tritici* Saito, *maydis* Bruderl, *delemar* (Boid) Wehmer and Hanzawa, *arrhizus* Fischer, *oryzae* Went and Pr. Geerligs, *nigricans* Ehrnb., *reflexus* Bainier, *artocarpi* Racib., and *microsporus* v. Tieg.

It has been shown [1] that the different species of Rhizopus do not all have the same optimum temperture for growth. Some species thrive at high temperatures, some at relatively low temperatures, and others at a temperature intermediate between the two extremes. Therefore, the 11 species studied have been separated into three groups with respect to their temperature relations. In all the experiments connected with the present investigations the same grouping of the different species has been observed, thus subjecting each organism to as nearly the optimum temperature for its growth as possible.

The cultures of *chinensis* were incubated at 40° C., those of *nodosus, tritici, maydis, delemar, arrhizus*, and *oryzae* at 30°, and those of *nigricans, reflexus, artocarpi*, and *microsporus* at 20°. Although so far as temperature is concerned the results are not strictly comparable, preliminary experiments showed that more reliable data could be obtained by growing the different organisms at temperatures suited to their growth than by subjecting them all to a uniform temperature. Some of the species, as for example *nigricans*, which requires a relatively low temperature, make no growth or only a feeble growth at 30° and none at 35°. On the other hand, *chinensis*, a high temperature form, makes a reduced growth at 30° and a feeble growth at 20°.

At the close of the incubation period (three days) the mycelial growth was lifted from the culture flask and the substrate was filtered through a fine grade of muslin. The mycelium was treated subsequently by acetone and ether according to the method previously described.[2] The solutions from the different flasks in which the same species had grown were made into a compound sample thoroughly shaken, and 25-cc. portions were used for maceration experiments. Likewise all the fungous felts of the same organism grown in the different flasks were brought together and treated as one sample, a weighed portion of the dried mycelium being used for maceration of the raw disks. Two types of controls were run with each set of experiments, as follows: (1) Sweet-potato decoction on which the fungus had grown for three days, which after the removal of the mycelium was steamed for 15 minutes to inactivate the enzym; (2) decoction which was identical with that used for

[1] Harter, L. L., Weimer, J. L., and Lauritzen, J. I. Op. cit.

[2] Harter, L. L., and Weimer, J. L. Op cit.

inoculation purposes but which had not supported a fungous growth. Maceration by the enzym in the solution and in the mycelium was carried out at a temperature of 40° C. for all the species. Before the addition of the raw sweet-potato disks the solutions and suspensions of the mycelium were held for one hour at 40° in order to bring them to the temperature at which maceration was to take place.

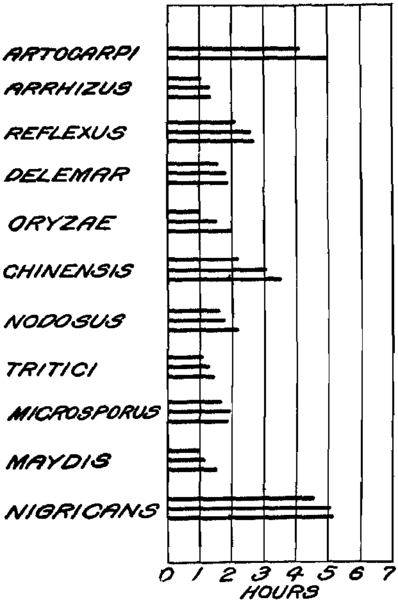


FIG. 1.—Graph showing the time required by the different *Rhizopus* species to completely macerat sweet-potato disks by the enzym in the solution on which the fungi had grown for three days; also tl comparative rate of maceration in the three experiments.

Maceration with the mycelium was carried out by the use of 0.5 gm ground in sand and suspended in 25 cc. of water. All the sweet potat disks (1 mm. thick and 1.5 cm. in diameter) required for maceration i the solution on which the fungus grew and in a water suspension of th mycelium were cut from a single potato for an entire experiment of a the species.

## EXPERIMENTAL DATA

The results obtained in the different experiments both as regards the maceration in the solutions and in a water suspension of the mycelium are represented graphically in figures 1 and 2. Each of the vertical lines of a single group represents the results obtained for a particular organism in a single experiment. The length of the vertical lines indicates the

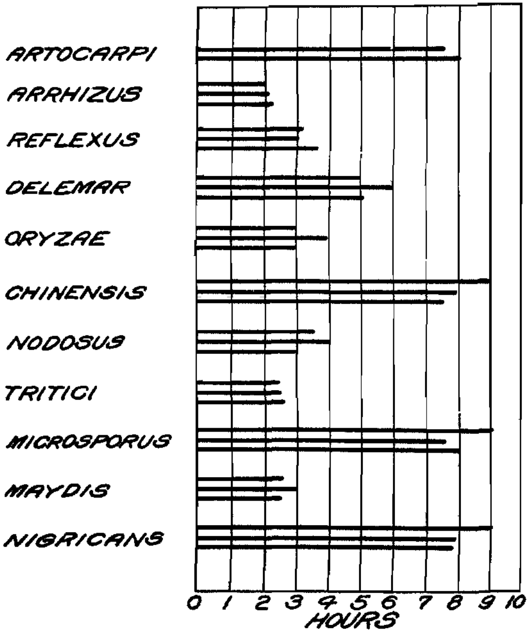


Fig. 1.—Graph showing the time required by the different Rhizopus species to completely macerate sweet-potato disks by the enzym contained in ⅓ gr. of the mycelium; also the comparative rate of maceration in the three experiments.

length of time in hours required to complete maceration of the disks, 1 cm. being equivalent to one hour. From these figures a direct comparison can be made of the results obtained from the different species as well as the variation in the results of the same species in different experiments.

## DISCUSSION OF RESULTS

### VARIATION

A comparison of the results as shown by figures 1 and 2 indicates that under the conditions of these experiments maceration was completed by the enzym exuded into the solution in a shorter length of time than by that contained in the mycelium. This difference, however, does not mean that the enzym is more powerful or more abundant in the solution than in the mycelium since no attempt was made to employ an amount of mycelium that would be equivalent in macerating power to the enzym of the solution. In these experiments maceration was regarded as complete when the disks pulled from opposite sides separated without any perceptible resistance. In completely macerated tissue coherence of the cells is entirely lost and the tissue can be readily pulped between the thumb and finger.

The data show that a considerable amount of variation exists in the results obtained in the different experiments with a single species both in respect to the solution and the mycelium. There are probably several factors responsible for these variations. In the first place a different supply of the sweet-potato decoction was prepared for each set of experiments. In spite of the fact that the different solutions were prepared to be alike as nearly as it is possible to make them, it can not be said, in view of the fact that different potatoes were used each time, that the various preparations were identical. After the cultures were inoculated incubation was carried out at a temperature which varied very little but possibly enough to influence slightly the rapidity and volume of growth. At the close of the incubation period the substrate and mycelium were handled as nearly alike as possible in all the experiments, but in spite of such precautions some variations might result. It would seem that the greatest source of error might be attributed to a variation in the composition of the potatoes from which the raw disks were cut. In this connection preliminary experiments showed that different potatoes are macerated in a different length of time the variations, however, being within relatively narrow limits. Furthermore, it is probable that the composition of the sweet potato is gradually changing with the increase in the length of time after digging. So far as their susceptibility to maceration is concerned it is interesting to note that a comparison between newly dug potatoes and those stored for several months showed that the latter are macerated in a shorter time than the former. Although the present experiments were carried out with a single variety the various experiments were conducted in sequence so that the later experiments were made on what might be termed older potatoes.

## COMPARISON OF SPECIES

### MACERATION OF DISKS IN THE SOLUTIONS

It appears from figure 1 that two species, *nigricans* and *artocarpi*, macerate raw sweet potato disks more slowly than any of the others, followed by *chinensis* and *reflexus* in the order mentioned. The other species complete maceration in a relatively short time, the most rapid being *arrhizus*, *tritici*, and *maydis*.

### MACERATION OF DISKS IN A SUSPENSION OF MYCELIUM

With respect to maceration by the mycelial enzym, four species, *nigricans*, *microsporus*, *chinensis*, and *artocarpi*, stand out as being conspicuously slow. So far as the mycelium is concerned *delemar*, a species intermediate between the slow and rapid forms, is less active than *reflexus* but more active than the latter when the solutions are used. Likewise the enzym contained in the mycelium of *microsporus* macerates slowly, while that in the solution, on the other hand, disintegrates the tissue rapidly. *Chinensis* shows a similar relationship existing between the enzym of the mycelium and that of the solution, although this species does not stand out as conspicuously as *microsporus*.

From the few illustrations cited it is evident that there is no complete correlation between the activity of the mycelial enzym and the activity of that exuded into the substrate. An examination of figures 1 and 2 shows that the different species do not secrete an equivalent amount of pectinase, since the completion of maceration by the enzym of both the mycelium and solution may vary greatly. It is conceivable and the results of these investigations seem to indicate that some species give up their enzym to the solution more readily than others. For example, the solution on which *microsporus* grew is relatively rich in pectinase while the enzym contained in the mycelium acts slowly. *Delemar* is another outstanding example of the same phenomenon.

## PECTINASE PRODUCTION IN RELATION TO PARASITISM

If a relationship between the production of pectinase by the different species of Rhizopus and their parasitism could be shown to exist, considerable light might be thrown on the physiology of parasitism, especially among fungi which are characterized by their ability to dissolve the middle lamella in advance of their growth. In a previous publication [1] it was pointed out that all the species of Rhizopus studied were parasitic on sweet potatoes with the exception of *microsporus* and *chinensis*. These two species were studied in connection with the others. They were given equal opportunity to cause decay, but in no case was there any evidence to indicate parasitism. However, both of these

[1] Harter, L. L., Weimer, J. L., and Lauritzen, J. I. op. cit.

species produced pectinase. The amount of pectinase in the mycelium at the end of the growth period was relatively small, but *microsporus* and to a lesser degree, *chinensis*, exuded enough into the culture solution to cause maceration in a much shorter time than either *nigricans* or *artocarpi*, both of which are parasites. Maceration of sweet-potato disks by means of the mycelial enzym of the two parasitic and nonparasitic species just mentioned was completed in about the same length of time. *Nigricans* is the most commonly isolated species and is probably responsible for most of the loss to sweet potatoes caused by this group of fungi. At a suitable temperature it decays sweet potatoes and other vegetables rapidly. The middle lamellae of sweet potatoes decayed by this species are dissolved some distance in advance of the growth of the mycelium, so that coherence is lost. In the early stages, at least, the cells themselves are not invaded by the fungus. The same may be said of *artocarpi*. However, in cultures *nigricans* and *artocarpi*, in contrast to the other species, exude a very small amount of enzym into the substrate and retain very little in the mycelium. *Delemar*, a species which readily decays sweet potatoes, seems to give up most of its pectinase to the substrate, so that maceration by means of the mycelium is relatively slow, at least within the limits of these experiments. All the other species are vigorous parasites, decaying the sweet potato within a few days under favorable conditions. They also produced large quantities of pectinase, relatively speaking, some of which is exuded into the solution and some retained by the mycelium, as shown by the fact that maceration, by both the mycelium and solution, is comparatively rapid.

SUMMARY

(1) The secretion of pectinase by 11 species of Rhizopus has been studied. All the species were found to produce pectinase and to exude some of it into the culture solution.

(2) The amount of pectinase produced varies with the species, grown under identical conditions. The mycelium of four species—*nigricans*, *microsporus*, *chinensis*, and *artocarpi*—and the solution on which two—*nigricans*, and *artiocarpi*—are grown is comparatively weak in pectinase. *Chinensis* and *microsporus*, whose mycelial enzym is weak, secrete it abundantly into the substrate.

(3) Two species, *nigricans* and *artocarpi*, both of which are parasitic on the sweet potato, secrete a relatively small amount of pectinase. On the other hand, *chinensis* and *microsporus*, two nonparasitic species, while retaining a small amount of enzym in the mycelium, secrete a comparatively large quantity of enzym into the culture solution.

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## HEMOTOXINS FROM PARASITIC WORMS

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### I. INTRODUCTION

Aside from the purely mechanical injuries which parasitic worms may inflict upon their host as a consequence of their migrations, displacement of a certain amount of the host's tissue, bites and laceration of the mucosa, obstruction of ducts, and various other mechanical disturbances, it has been generally assumed that they may also produce harmful effects as a result of their toxic secretions. Despite the fact that the data on which the view that parasites secrete toxic substances is based, so far as they have been recorded heretofore in the literature, are somewhat contradictory, they have been accepted by a large number of investigators as affording a more plausible explanation of the chemical pathology of helminthiasis than the data with reference to any other theory that has thus far been advanced. With reference to the subject of toxic products of parasitic worms, Wells (*1918*)[2] states:

> The subject has received much less consideration than its importance deserves, as we are quite in the dark as to how much of the effects produced by animal parasites are not merely mechanical, but are due to soluble poisons that they secrete or excrete. Some of the parasites probably cause harm mechanically and in no other way, but with most of them there is more or less evidence of the formation of poisonous substances.

While it must be admitted that the evidence in favor of the view that parasites secrete products toxic to the host is as yet rather incomplete, the fact of the existence of such toxic products can not be denied. So far as they have been investigated, the serological reactions of hosts harboring parasites afford proof that parasitic worms liberate products against which the host develops defense or "immunity" reactions. It has been known for a relatively long time that in cases of infestation with species of Trichinella, Schistosoma, Necator, and Anyclostoma a

[1] Resigned December 15, 1920.

[2] Dates in parenthesis refer to "Literature cited," p. 428–432.

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high eosinophilia is commonly present. An increase in the number of eosinophile leucocytes has also been observed, although not as regularly, in cases of infestation with species of Ascaris, Oxyuris, Strongyloides, and other nematodes. Similar conditions have also been encountered in cases of infestation with *Taenia solium*, *T. saginata*, *Fasciola hepatica*, *Clonorchis sinensis*, and other cestodes and trematodes. As a matter of fact, eosinophilia is so commonly associated with parasitic infestation that the finding of a high eosinophile content in the peripheral blood is generally considered as presumptive evidence of parasitic infection. In a recent extensive review of the literature on the subject of eosinophilia, Schwarz (*1914*) states that an increase in the number of eosinophile leucocytes in the peripheral circulation in cases of parasitic infestation is, from an etiological viewpoint, the most clear-cut illustration of general eosinophilia.[1]

Aside from the cellular immunity reactions, as shown by the increase in the number of eosinophile leucocytes in the blood in cases of infestations with parasitic worms, there appears to be evidence of a humoral immunity as well. In the case of hydatid (Echinococcus) disease of man and animals, it has been shown by a number of investigators that specific antibodies are present in the blood of the host, demonstrable by the technic of complement fixation and precipitate formation. That such immunity reactions are not limited to hydatid disease is the opinion of certain investigators, who support their views by experimental evidence which shows that specific antibodies are also present in cases of infestations with species of Ascaris, Fasciola, Schistosoma, and other parasitic worms.[2]

The facts cited in the preceding paragraphs appear to indicate that hosts harboring parasitic worms develop typical defense or "immunity" reactions to the absorption of foreign and presumably toxic substances of parasitic origin. A logical corollary to the study of the serological reaction of animals to secretions of parasitic worms is the study of the secretions themselves with reference to their chemical and physiological properties. This subject has recently received considerable attention in studies on the causes of pernicious or infectious anemia of horses, a disease of unknown etiology, which Seyderhelm and Seyderhelm (*1914*) attribute to a secretory product of an internal parasite (the larvae of Gastrophilus). Although the assumption of the Seyderhelms has not been confirmed, numerous experiments by different investigators have shown that injection into animals of extracts of various parasitic worms may lead to serious consequences, frequently terminating in death. Despite the fact that these experiments are in a general way confirmatory of the work of earlier investigators on the physiological effects of extracts of

[1] "Die Vermehrung der α-Zellen in peripheren Blut bei Anwesenheit von Parasiten aus dem Stamme der Würmer ist vielleicht die ätiologisch am meisten klargestellte Form der allgemeinen Eosinophilie."

[2] References to and a summary of this phase of the subject may be found in an article by O. Ghedini.

parasitic worms, the experimental evidence on the subject is somewhat contradictory, due no doubt to the fact that different investigators experimented under different conditions. The study of the effects of extracts of parasites on living animals presents numerous difficulties and complications and may lead to contradictory results unless suitable precautions are taken to control various extraneous factors. More accurate studies on the effects of toxic products may be carried out in vitro provided the toxic substance in question has affinity for tissues and cells available for such experiments. As is well known, red blood cells serve as excellent indicators of test-tube reactions in which hemotoxic substances are involved, and in the case of toxic products of parasitic origin experiments with red blood cells are of great importance in view of the fact that in many parasitic infestations anemia is a characteristic symptom. The effects of extracts of parasitic worms on red blood cells especially of extracts of those parasites that are known to cause anemia are thus of interest with reference to the possibility that the parasites in question secrete specific toxins for the blood (hemotoxins).

## II. REVIEW OF LITERATURE ON HEMOTOXINS IN PARASITIC WORMS

The same year in which Ehrlich (*1898*) announced the discovery of a blood toxin produced by *Bacillus tetanus*, Schaumann and Tallqvist (*1898*) reported the discovery of a blood toxin in the broad tapeworm of man (*Diphyllobothrium latum*). Ehrlich's discovery in the field of bacteriology served as a stimulus to the study of bacterial hemolysins by various investigators and was followed by a series of investigations into the nature and action of these hitherto unknown products of bacterial growth. Although the discovery of Schaumann and Tallqvist did not arouse the same degree of activity in parasitology as Ehrlich's discovery aroused in bacteriology, the results of their investigations were not without influence on subsequent researches in parasitology, the influence being especially marked in connection with studies on the causes of the anemia that occurs in cases of infestation with hookworms.

The facts published by Schaumann and Tallqvist (*1898*) may be briefly summarized as follows:

Macerated material of *Diphyllobothrium latum* contains a hemolytic substance active in vitro as well as in vivo. Peptic digestion liberates the hemolysin from the tissues of the parasites. The introduction of *D. latum* material into dogs parenterally or per os leads to a marked reduction in the number of erythrocytes.

In a later paper Tallqvist (*1907*) gives a more detailed account of the nature of the hemotoxic secretions of *Diphyllobothrium latum*. The hemolytic principle is closely bound to the cells of the parasite and is but slightly soluble in water and physiological salt solution. By means of peptic digestion and alcohol or ether extraction it becomes dissociated from the tissues and goes into solution. The hemolysin is thermostabile and does not cause the development of antibodies when injected into animals. In these respects it resembles normal tissue hemolysins. Tallqvist found, moreover that *D. latum* contains not only a hemolysin but also a hemagglutinin. The latter is soluble in water in contrast to the water-insoluble lipoidal hemolysin. The hemagglutinin as well as the hemolysin is nonspecific. The potency of these agents varies however, for different species of red blood corpuscles.

Faust and Tallqvist (*1907*) studied the Diphyllobothrium hemolysin as to its chemical nature. These investigators found that extraction of the entire worm material in ether removed all the hemolysin from the tissues of the parasite, since the removal of

the ether-soluble fraction left a fraction entirely devoid of hemolytic activity. The ether-soluble fraction was then freed from its lecithin and cholestrin content without injuring its hemolytic activity. In the remaining ether fraction (freed from lecithin and cholesterin) Faust and Tallqvist identified three fatty acids, namely, palmitic, stearic, and oleic acids. The first two substances did not exhibit any hemolytic properties, whereas oleic acid was found to be markedly hemolytic. These investigators therefore concluded that oleic acid is the active principle of Diphyllobothrium hemolysin.

In a later paper Faust (*1908*) records the results of experiments on the effects of oleic acid on dogs when introduced per os. In brief, this investigator concluded that prolonged feeding of oleic acid to dogs produced anemia in the latter, as evidenced by a reduction in the number of red blood corpuscles. Beumer (*1919*), however, has found, on the contrary, that animals may be fed daily with considerable quantities of oleic acid for long periods without permanent ill effects, and has failed to substantiate the harmfulness of oleic acid.

In this connection it is of interest to recall the experiments of Dascotte (cited by Weinberg, *1912*), who states that extracts of *Taenia solium* and *T. saginata*, cestodes parasitic in man, dissolve human red blood corpuscles. Dascotte found, moreover, that the hemolysin from these parasites is soluble in alcohol and resistant to heat at temperatures of 100° to 120° C. Calamida (*1901*) found that extracts of two species of cestodes from carnivores (*Dipylidium caninum* and *Multiceps multiceps*) are hemolytic to the red blood corpuscles of rabbits and guinea pigs and that the hemolysin goes through the pores of a Berkefeld filter. According to Weinberg (*1907*), physiological salt-solution extracts of two species of tapeworms parasitic in horses (*Anoplocephala plicata* and *A. perfoliata*) have no deleterious effects on the blood corpuscles of the horse. Tallqvist (*1907*), on the other hand, denies the presence of hemolysins in cestodes other than *Diphyllobothrium latum*. He states that he worked with a number of species, including *T. saginata*. He admits that he sometimes observed slight hemolytic effects of extracts of these parasites but expresses the opinion that they are to be ascribed to secondary degeneration products associated with acid formation.

While *Diphyllobothrium latum* is capable of causing severe anemia in man, clinically indistinguishable from pernicious anemia and, according to many investigators, differing from the former in one respect only, namely, by the disappearance of the symptoms and recovery of the patient after expelling the parasite, there are numerous cases on record in which the presence of *D. latum* in man was not accompanied by anemia. In fact, grave blood disturbances in cases of *D. latum* infection are, according to the observations on record, not as common as the incidence of infection with this tapeworm, a fact which has given rise to considerable speculation as to the causes of the occasional appearance of anemia in the course of infection with this parasite. These speculations will be referred to elsewhere in this paper.

In contrast to the occasional appearance of anemia in cases of infection with *Diphyllobothrium latum* infections with hookworms (Necator and Ancylostoma) are generally accompanied by severe anemia. That the causes of anemia in hookworm disease are due to a toxin is a view which was adopted by a number of investigators on a purely a priori basis, because the direct abstraction of blood by these parasites, even when present in large numbers, fails to account for the severity of the clinical picture usually present in such cases. This fact was recognized early in the study of the disease, and led to the postulation of the "toxin theory."

Luscana (*1890*) found that as a result of injecting rabbits with urine taken from patients suffering from hookworm disease, the animals developed symptoms of anemia. It was not until 1905, however, that the toxin theory received more direct experimental support from Calmette and Breton. These investigators found that salt-solution extracts of the Old World hookworm of man (*Ancylostoma duodenale*) are hemolytic to the red blood cells of man. Alessandrini (*1904*) had already found by direct microscopic

observation that human red blood corpuscles are destroyed when placed in contact with the cervical glands isolated from hookworms (species not given but presumably *A. duodenale*), but subsequent investigation showed that the hemolysin is not limited to the cervical glands.

Loeb and Smith (*1904*) in the course of experiments with salt-solution extracts of the dog hookworm (*Ancylostoma caninum*), found that these extracts showed no hemolytic properties and left the blood still intact and uncoagulated after being in contact with it for 17 hours. Reference to the work of these investigators on the anticoalgulating property of hookworm extract (*A. caninum*) will be made elsewhere in this paper.

Liefmann (*1905*) found that in two out of three experiments salt solution extracts of *Ancylostoma caninum* produced slight hemolysis of dog blood. This writer observed intact erythrocytes in the intestines of the worms and therefore came to the conclusion that the parasites do not secrete a hemolysin. Liefmann fails to state whether or not he washed the blood corpuscles before testing them against hookworm extract.

Preti (*1908*) found that the Old World hookworm of man (*Ancylostoma duodenale*) contains a hemolysin insoluble in salt solution but soluble in ether and alcohol. He states that tryptic digestion liberates the hemolysin and renders it soluble in water. He found the hemolysin to be resistant to boiling for three hours and nonspecific, since it was equally potent against the blood corpuscles of several other species of animals as well as man.

In the course of his investigations concerning ancylostomiasis and beriberi, Noc (*1908*) found that physiological salt-solution extracts of the hookworm of man (*Necator americanus*) are hemolytic to the washed red blood corpuscles of man. He states that the hemolysin withstands a temperature of 80° C. for one hour without injury to its potency. Noc found that whereas the blood serum of patents suffering from severe ancylostomiasis and beriberi contained no antihemolysins, that of normal persons and of those recovering from these diseases was antihemolytic.

De Blasi (*1908*) examined the blood serum of 12 human subjects infested with hookworms (*Ancylostoma duodenale*) and found that after the serums were heated for 30 minutes at 56° to 62° C. they acquired hemolytic properties. Before heating, the serums in question were not hemolytic. Heating the serum evidently destroyed some antibodies which neutralized the potency of the hemolysin. The heated serum of normal persons, according to this writer, did not contain any hemolysins.

Whipple (*1909*) records tests of salt-solution extracts of *Ancylostoma caninum*, *A. duodenale*, and *Necator americanus* on unwashed citrated blood of man, dog, and rat. He states that he found a weak hemolysin in the three species of hookworms exhibiting similar properties, namely, nonspecificity, susceptibility to boiling which destroys it, and distribution in all parts of the body of the worms. According to Whipple, the hemolysin is only demonstrable in concentrated extracts, and probably bears no relation to the secondary anemia of ancylostomiasis.

Loeb and Fleisher (*1910*) state that a salt-solution extract of *Ancylostoma caninum* containing as much as 5 mgm. of the powdered worm material in 1 cc. of salt solution did not produce any hemolytic effect on the washed erythrocytes of the dog. These writers also state that lecithin used in doses in which it alone produced no hemolytic effect failed to activate *A. caninum* extract. Loeb and Fleisher admit the possibility that the temperature at which the specimens were dried (42° to 50° C.) may have had an injurious effect on the hemolysin, but they do not consider this very probable.

Recently Usami and Mano (*1919*) have studied the effects of hookworm extracts on red blood cells. These writers state that hookworm hemolysin is thermostabile, insoluble in water, and soluble in alcohol, ether, and acetone.

It will be seen from the foregoing summary with reference to hookworm extracts that Loeb and Smith (*1904*) and Loeb and Fleisher (*1910*) are the only investigators who failed to observe hemolysis in the presence of these extracts. As will be shown elsewhere in this paper, the negative results of Loeb and Smith may have been due

to the antilytic action of normal blood serum. The negative results recorded by Loeb and Fleisher (*1910*) may have been due to insufficient or faulty extraction of the worm material, insufficient quantity of powder used in the experiments, or possibly to the destruction of the hemolysin by drying at temperatures between 42° and 50° C. The results recorded by Preti (*1908*) as regards the insolubility of the hemolysin in salt solution and its resistance to boiling are at variance with those of other investigators, and, as will be shown in the following pages, are not in harmony with the results obtained by the present writer. Moreover, Preti's results can not be accepted as conclusive, owing to his failure properly to control his experiments. Alessandrini's attempt (*1904*) to associate the secretisn of hemolysin with the cervical glands of the parasites is not sustained by Whipple (*1909*), who found the hemolysin in all parts of the worm.

It is interesting to observe that the different species of hookworms referred to in the foregoing summary have the common biological property of secreting a substance destructive to red blood cells. Inasmuch as hookworm disease is characterized by severe anemia, the presence of a blood-destroying substance in the parasites is highly significant.

In addition to the hemolytic substance which is present in hookworms, Loeb and his collaborators have shown that the hookworm parasitic in dogs (*Ancylostoma caninum*) also secretes a substance which inhibits coagulation of blood in vitro (Loeb and Smith, *1904;* Loeb and Smith, *1906;* Loeb and Fleisher, *1910*). The results of experiments by these investigators with reference to the anticoagulins of hookworms may be summarized as follows: In *A. caninum* a substance is present which retards coagulation of blood in vitro. This substance which is present in the anterior part of the worm and practically absent in the posterior part is not destroyed but is markedly weakened by boiling for 15 minutes. The substance does not resemble hirudin, a toxic constituent of the leech, but appears to resemble cobra venom so far as its physiological properties are concerned. It is of interest to note in this connection that Liefmann (*1905*), who rejects the view that the hookworm secretes a hemolysin, likewise rejects the view that this parasite secretes an anticoagulin, since he obtained positive results in but one out of three experiments which he performed. Liefmann ascribes his positive results to substances from the intestine which may have adhered to the worms, namely, pancreatin and peptone. Loeb and Smith (*1906*) point out, however, that in view of the fact that they washed the worms carefully and that neither peptone nor pancreatin is known to inhibit coagulation of dog's blood in vitro, and further, in view of the fact that the posterior parts of the hookworms showed but a slight anticoagulating effect on dog blood and that extracts of ascarids and tapeworms from dogs did not retard the coagulation of dog blood, Liefmann's contention can not be sustained.

The carefully controlled experiments of Loeb and his collaborators leave no room for doubt as to the presence of a hemotoxin in *Ancylostoma caninum* which inhibits the coagulation of dog blood. Loeb and Smith ascribe etiological significance to this toxin and believe that it has the power of causing small hemorrhages in regions of the intestine that have been lacerated by the worms.

The pathological rôle of the whipworm (*Trichuris trichiura*) parasitic in man is emphasized by Askanazy (*1896*), who states that this parasite feeds on blood, basing his assertion on the presence of iron pigment in the intestine of the worm demonstrable by the Berlin blue reaction. Askanazy assumed, of course, that the iron found in the worm is obtained from the hemoglobin of the host's blood. Schultze (*1905*) rejects Askanazy's interpretation and considers that the pigment in question is obtained from the host's intestine rather than from the blood.

Guiart (*1908*) presents conclusive evidence as regards the bloodsucking habit of *Trichuris trichiura*, since he found blood-engorged specimens in a human patient. Guiart's observation has been confirmed by a number of investigators, including Garin,

Seidelin, and Leon (Guiart, *1914*). Guiart and Garin (*1909*) found that the presence of Trichuris eggs in the feces of human subjects is correlated with the presence of blood in the feces as shown by a positive Weber test.

As to the presence of hemotoxic secretions in whipworms, Whipple (*1909*), who experimented with extracts of these parasites, found that they contained a hemolytic substance destructive to the red blood cells of the dog and of man. Whipple states that the hemolysin left some samples of human red blood cells intact but was destructive to others. Garin (*1913*) performed similar experiments with Trichuris extracts and confirmed the presence of a hemolysin in these parasites. According to Garin, the whipworm hemolysin is thermostabile, being destroyed by 30 minutes' heating at 56° C. The inactivated hemylosin can not be reactivated by normal guinea-pig serum (complement), according to this investigator. Garin states, furthermore, that whereas he obtained positive results with human red blood corpuscles the results of experiments with the erythrocytes of rabbits and guinea pigs were doubtful.

A survey of the literature relating to the pathogenic rôle of *Ascaris lumbricoides* reveals the fact that this parasite may be responsible for anemia, which is sometimes mistaken for hookworm anemia or for pernicious anemia. The clinical reports of Demme (*1891*) have become a classic illustration of this fact. In brief, Demme found a child suffering from severe intestinal catarrh, with a high-grade pernicious anemia showing a red blood count of 2,450,000 and a hemoglobin content of 40 per cent. Two weeks after numerous worms (*A. lumbricoides*) had been expelled from the child's intestine the red blood corpuscle count rose to 4,200,000 and the hemoglobin content reached 70 per cent. In a second case of apparent pernicious anemia, which resulted in death and in which the erythrocytes had diminished to 1,650,000 per cubic millimeter, numerous ascarids were found on post-mortem examination which were apparently responsible for the death of the child. Kuttner (*1865*) found that in a girl aged 12 blood destruction occurred and that this was cured by expelling a number of ascarids. According to Filatoff (*1897*), Karaven cured a case of pernicious anemia in a child by expelling a number of ascarids from its intestine. François (*1906*), in the course of his investigations on anemia of miners, found many cases of severe anemia in which hookworms were not present but which showed numerous Ascaris eggs in the feces. A number of observations by different investigators on hogs and horses infested with ascarids and on man infested with *A. lumbricoides* bear out the fact that symptoms of anemia are frequently associated with such infestation.

As to the manner in which species of Ascaris cause anemia two views have been advanced, which are not mutually exclusive. Guiart (*1899*), who accepts the view that worms of this genus secrete a hemolysin, inclines strongly to the view that they also lacerate the mucosa, thus causing hemorrhages. In support of this view Guiart describes and figures *Ascaris conocephala* attached to the stomach of a dolphin, the head of the parasite being deeply embedded in the mucosa. Guiart refers to the observations of Leroux, who found lesions in the intestine of a human being infested with ascarids resembling lesions produced by ascarids on the mucosa of the dolphin. Friedberger and Fröhner (*1895*) also support this view and state that dogs that harbor numerous ascarids show on post-mortem examination of the intestine numerous round, dark spots, surrounded by an inflamed zone, due, in their opinion, to bites of the worms. According to Garin (*1913*), several observers, including Weinberg, have found ascarids attached to the mucosa. Garin admits, however, that despite the fact that he made numerous post-mortem examinations of human subjects infested with *A. lumbricoides* and of dogs and cats infested with ascarids, in the latter cases shortly after death, attached parasites were never observed by him. He confirms, however, the presence of reddish points surrounded by an ecchymotic area in the mucosa of the intestine of infested subjects, both human and animal. Thaler (*1918*) has recently reported a case of persistent intestinal hemorrhages in a human subject which did not

yield to symptomatic treatment and which was cured only after removing several ascarids.

The view that Ascaris secretions contain hemotoxins was first advanced by Schimmelpfennig (*1902*), who found that in the presence of the coelomic fluid of *Ascaris equorum* red blood corpuscles of the horse became crenated and were ultimately destroyed. Schimmelpfennig furthermore discovered oxyhemoglobin in the coelomic fluid of the parasite, a fact which led him to regard this worm as a bloodsucker. Weinberg (*1907*), Whipple (*1909*), and Alessandrini (*1913*) failed to observe any toxic effect of salt-solution extracts of species of Ascaris on red blood cells. Flury (*1912*), on the other hand, records the presence of strong hemolysins in the coelomic fluid of species of Ascaris. Flury ascribes the hemolytic action of Ascaris secretions to free fatty acids, of which oleic acid is the most active principle. In the course of his studies on the pharmacology of salt-solution extracts of worms of the genus Ascaris, Brinda (*1914*) found that injection of the extracts into guinea pigs brings about a reduction in the number of erythrocytes and a diminution in the hemoglobin content of the blood. Recently Shimamura and Fujii (*1917*), in the course of their investigations on "askaron," a toxic constituent of worms of the genus Ascaris, state that ether-soluble and alcohol-soluble fractions of Ascaris material contain a hemolytic agent. The present writer (Schwartz, *1919*), in a preliminary paper on the hemolytic effects of Ascaris extracts, has briefly described the properties of the hemolysin.

A number of investigators have found, moreover, that the coelomic fluid of worms belonging to the genus Ascaris contains a substance that inhibits the coagulation of blood. Weil and Boyé (*1910*) found that as a result of injecting the fluid of *Ascaris equorum* into rabbits the blood of the latter when drawn remains uncoagulated for 20 minutes longer than blood from a normal rabbit. Experiments with rabbit blood and Ascaris fluid in vitro yielded negative results, according to these investigators. Leroy (*1910*) likewise observed that the blood of dogs which had been injected with the body fluid of *A. equorum* coagulated more slowly than blood from normal dogs. Flury (*1912*) observed that Ascaris fluid delayed the coagulation of dog blood and of human blood in vitro. That Loeb and Smith (*1904*) failed to observe anticoagulins in extracts of dog ascarids that are active in vitro has already been mentioned.

Worms belonging to the genus Strongylus (frequently referred to as Sclerostomum) are parasitic in the large intestine of horses. These nematodes attack the mucosa, to which they may be found adhering by means of their buccal capsule. In view of the fact that these parasites somewhat resemble hookworms in their attacks on the intestinal mucosa and in the effects which they produce on the host, Weinberg (*1907*) investigated their hemotoxic secretions primarily with a view of throwing light on the causes of anemia due to hookworms. This investigator found that physiological salt-solution extracts of freshly collected Strongylus material dissolves erythrocytes of horses, cattle, sheep, rabbits, and guinea pigs. The parasites secrete, therefore, a nonspecific hemolysin. Weinberg determined that the hemolysin is thermostabile, resisting heat at a temperature of 115° to 120° C. for 15 to 20 minutes. In addition to the hemolysin, Weinberg found that these parasites secrete a substance which inhibits the coagulation of horse blood in vitro. He also found that salt-solution extracts of worms of the genus Strongylus contain a substance which when brought in contact with the blood serum of the horse causes the formation of a precipitate. The precipitin, too, is nonspecific in its action, since it was found by Weinberg that it produces a precipitate when added to rabbit-blood serum.

Bondouy (*1908*, *1910*) studied the chemical composition of worms belonging to the genus Strongylus, with special reference to their hemolytic constituents, and confirmed in the main the results obtained by Weinberg as regards the presence of a soluble hemolysin in these parasites. The new facts discovered by Bondouy may be briefly summarized as follows: The parasite contains soaps and free fatty acids which exert a destructive effect on red blood cells in vitro. Bondouy states, however, that the

presence of these substances in the parasite is due to its blood sucking habit, basing his assertion on the fact that blood serum contains neutral fats, fatty acids, and soaps. This writer found a lipolytic enzym in worms of the genus Strongylus which apparently converts the storage fat into fatty acid. It is of interest to note also that Bondouy found neither lecithin nor cholesterin in the parasite. Lecithin, as is known, has the property of activating certain hemolytic agents, namely, snake venoms, whereas cholesterin inhibits hemolysis of blood by active hemolysins. Contrary to Weinberg's experience (Weinberg, *1907*), Bondouy found that Strongylus hemolysin is soluble in alcohol. From the alcohol-soluble fraction of the parasite this writer isolated an extremely active hemolysin which he identified as an alkaloid. He also found a ptomain in the parasites which exhibited hemolytic properties.

Brumpt and Joyeux (quoted by Brumpt, *1910*) found that a watery extract of the stomach worm of sheep (*Haemonchus contortus*) produced a slight hemolytic effect[1] after 2¼ hours and a total hemolysis after 12 hours. Cuillé, Marotel, and Panisset (*1911*) state that extracts of sheep strongyles (species, of which apparently several were involved, not given) did not exert any effect on sheep red blood corpuscles from either healthy or sick animals. These writers also state that extracts of these parasites contained hemoglobin.

According to Garin (*1913*) *Graphidium strigosum* and *Trichostrongylus retortaeformis*, nematodes parasitic in the stomachs of hares and rabbits, secrete hemolysins. With reference to the hemolysin of *G. strigosum*, Garin found that it is secreted by the living worm in vitro. He found, furthermore, that the hemolysin is apparently a complex substance and acts on the blood not directly but in combination with complement. Heating at 55° C. for 30 minutes does not destroy but merely inactivates the hemolysin, which may be reactivated by normal serum, according to this investigator. In view of the limited number of experiments which Garin performed, his conclusions can be accepted only with reservation. The work requires confirmation. As for the hemolysin from *T. retortaeformis*, Garin found it to be far less potent than that of *G. strigosum*. He also states that the hemolysins from the two species have far greater affinity for the blood cells of rabbits than for those of other species of animals and are therefore relatively specific.

Yagi (*1910*) found that salt-solution extracts of the blood fluke, *Schistosoma japonicum*, are hemolytic to erythrocytes of cattle, sheep, and rabbits. He found, furthermore, that this hemolysin is soluble in ether and concluded that it is probably a fatty acid. Yoshimura (*1913*) experimented with salt-solution extracts of the same species and found them to be destructive to rabbit erythrocytes. Human blood cells, according to this writer, are refractory to these extracts. Yoshimura also experimented with ether extracts, which he found destructive to rabbit red blood corpuscles and to a lesser extent destructive to human red blood corpuscles.

According to Guerrini (*1908*), *Fasciola hepatica* secretes a hemolysin which is absorbed by the host and is demonstrable in the blood serum of the latter.

Alessandrini (*1913*) records the results of experiments with extracts of *Macracanthorhynchus hirudinaceus*, the thorn-headed worm of the hog. He tested the body fluid and extracts of various parts of the worm and found them to be destructive to the red blood cells of swine, cattle, and sheep. Alessandrini states that the hemolysin from *M. hirudinaceus* is a colloidal substance insoluble in alcohol, soluble in water, and highly sensitive to heat, since a temperature of 40° C. diminished its potency and a temperature of 55° destroyed it entirely.

Although the larvae of species of Gastrophilus which occur in the stomach of the horse are in a zoological sense not parasitic worms, the results of a study of their toxic secretions may be included in this review because these larval parasites are biologically more closely related to parasitic worms than they are to free-living insect larvae. At any rate their secretions may be absorbed by the host and give rise to disturbances

[1] No details are given as to kind and quantity of blood corpuscles used.

similar to those produced by the secretions of helminths. Weinberg (*1908*) investigated the hemotoxic properties of the fluid of these parasites and obtained the following results: Extracts of the intestine and of the red cells of the fatty bodies of the larvae contain a soluble hemolysin, nonspecific in its action and susceptible to heating for ½ hour at 56° C., which does not destroy it but merely weakens its potency. Weinberg found, moreover, that these extracts have an inhibiting action on the coagulation of the blood of several species of animals.

## SUMMARY

Summarizing the results of hitherto recorded investigations on hemotoxins from parasitic worms, it may be stated that while there is more or less contradictory evidence in the literature the following facts have apparently been established:

1. Certain parasitic worms secrete substances that affect the blood of their host deleteriously. These substances, which may be designated as hemotoxins, are in general nonspecific in the sense that they are also active toward blood of animals other than their normal host.

2. *Diphyllobothrium latum*, a tapeworm which is known to cause severe anemia, contains a hemolytic agent. It appears questionable that this agent is oleic acid, as claimed by Faust.

3. Concerning hemolysins in cestodes other than *Diphyllobothrium latum* no definite conclusions can be drawn from the literature on the subject, but that hemolysins are present in several species appears probable.

4. *Schistosoma japonicum* secretes an ether-soluble hemolysin.

5. Hookworms (Ancylostoma and Necator) secrete a hemolysin and an anticoagulin.

6. Whipworms (*Trichuris trichiura*) apparently secrete a hemolysin.

7. Worms belonging to the genus Ascaris contain a hemolysin which is closely bound to the tissues of the worms and is therefore but slightly soluble in water, which fact accounts for the negative results obtained by certain investigators. These parasites also appear to secrete a feeble anticoagulin.

8. Worms of the genus Strongylus secrete a hemolysin and an anticoagulin. The hemolytic principle of these parasites is apparently an alkaloid, although other substances found in them show hemolytic power.

9. *Haemonchus contortus* apparently secretes a weak hemolysin.

10. Extracts of *Macracanthorhynchus hirudinaceus* are apparently destructive to erythrocytes.

11. Hemolytic and anticoagulating properties are found in extracts of the larvae of species of Gastrophilus.

12. Hemolytic substances from parasites are soluble in alcohol[1] and ether, thus resembling lipoids.

13. With respect to their resistance to heat, hemolysins from animal parasites vary, but in general they are thermostabile.

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[1] According to Alessandrini the hemolysin from *Macracanthorhynchus hirudinaceus* is insoluble in alcohol.

Owing to the fact that the direct abstraction of blood by parasites appears to be inadequate as an explanation of the causes of anemia in parasitic diseases, and in view of the fact that in tapeworm infections which are accompanied by anemia due entirely to the presence of the parasites the direct abstraction theory is inapplicable, the view that hemolysins from parasites are of etiological significance in parasitic diseases appeared to be entirely justified.

III. TECHNIC

Unless otherwise indicated, the experiments described in the following pages were performed with washed red blood cells. In most cases the blood was defibrinated, filtered through gauze, centrifuged to remove the serum, and washed in physiological salt solution at least three times to free it from traces of serum. In a few cases a somewhat different procedure was followed. The blood was collected in a 2 per cent solution of sodium citrate or in physiological salt solution containing 1 per cent sodium citrate. The removal of the serum and subsequent washing in physiological salt solution were carried out as in the case of defibrinated blood. Unless otherwise stated, a 5 per cent suspension of corpuscles, made by suspending 1 part of washed red blood corpuscles in 19 parts of physiological salt solution, was used.

Blood serum used in these experiments was obtained as follows: In the case of rabbits blood was obtained by severing the marginal ear vein, and in the case of the larger domestic animals it was obtained at an abattoir from animals that were being bled and was allowed to drop into a sterile centrifuge tube. The tube containing the blood was allowed to remain at room temperature for a few hours. By means of a sterile platinum wire the clot was loosened from the sides of the tube to which it adhered and the tube was then centrifuged. The clear serum was pipetted off, and if the serum was to be kept for more than three days sufficient phenol was added to give a phenol content of 0.25 to 0.5 per cent; otherwise no preservative was added.

Extracts of parasites were made from fresh material and from dried material. In both cases the living specimens were obtained shortly after they had been removed from the host. Certain writers who deny the presence of toxic substances in parasitic worms base their objection to the evidence in favor of the view that parasitic worms secrete toxic substances on the grounds that extracts are frequently made from parasites that are obtained as a result of anthelmintic medication and that the toxicity may be due to traces of anthelmintic which adhere to the surface of the parasite or to secondary degeneration products of dead worms. The present writer has been careful to use fresh specimens in order to avoid complications of the sort just mentioned. It should also be stated that the parasites obtained from the intestines and other organs were washed in physiological salt solution and were transferred three or

four times in succession to fresh salt solution. In this manner the surface of the worms was freed from adhering intestinal material. In the case of salt-solution extracts that were allowed to remain at room temperature or in an incubator for several hours or for a few days, a preservative, usually a few drops of chloroform, was added to the extract to inhibit bacterial growth.

Specimens were dried as follows: After having been washed a number of times in physiological salt solution, the surface of the worms was dried with filter paper. The specimens were then placed in a single layer in a glass dish and allowed to dry either at room temperature in an incubator or in vacuum over sulphuric acid. Small worms dry in a few hours, even at room temperature, and become sufficiently crisp to be pulverized. Larger specimens dry more slowly and are usually crisp in about 48 hours. The dried material was triturated in a mortar and stored in bottles, usually in a dark place.

Special points in technic are covered in connection with the different series of experiments and are not taken up in this connection.

As used in this paper, the terms physiological salt solution and salt solution refer to an 0.85 per cent solution of sodium chlorid in distilled water.

Controls on all samples of blood corpuscles used in the experimental work described in the following pages were maintained in connection with each experiment or series of experiments.

## IV. EXPERIMENTS WITH HEMOLYTIC EXTRACTS OF ASCARIS LUMBRICOIDES

### 1. METHOD OF OBTAINING FLUID FROM WORMS

Body fluids and extracts were obtained from specimens of *Ascaris lumbricoides* from swine. A supply of these parasites is available in abattoirs during all seasons of the year.

The fluid which is present in the body of the worms was usually obtained by cutting off the posterior end of medium-sized to large-sized specimens and allowing the pinkish liquid to drop into a test tube. Fluid obtained in this manner does not keep well and is available only for immediate use. Allowed to stand, even at a low temperature, the body fluid thus collected undergoes bacterial decomposition in about 24 to 36 hours. Weinberg and Julien (*1911*) describe a method of collecting Ascaris body fluid under aseptic precautions. Briefly, the method consists in drying the worms with filter paper, holding the ends of each specimen and passing the middle region of the worm through the flame of a Bunsen burner until the cuticle bursts. The first two or three drops of fluid which ooze out are discarded and the remaining fluid is allowed to drop into a sterile tube. This procedure was tested by the present writer with inconstant results so far as the keeping qualities of

the fluid were concerned. In some cases sterile fluid was obtained in this manner, but more often the fluid became contaminated. The contamination was extraneous and not inherent in the body fluid of the worms, since a number of experiments performed by the writer showed quite conclusively that the intact body fluid of Ascaris is sterile.

In the course of the experimental work described in this paper specimens were kept alive in vitro for a few days. This necessitated information as to the conditions that are favorable to the survival of the parasites outside of the host. The customary procedure of keeping parasitic worms at a low temperature is not applicable to *Ascaris lumbricoides* when considerable periods, generally in excess of 24 hours, are involved. Incubator temperatures (37.5°C.) are more favorable than refrigerator temperatures, but so far as longevity of the worms outside the host is concerned, a temperature ranging from above 25° to 32° was found to be the most favorable. The worms were kept in shallow dishes and in beakers, and sufficient salt solution was added to cover the worms. Fluid from worms that had thus been subjected to starvation was obtained in the same manner as fluid from fresh worms.

## 2. EXPERIMENTS WITH THE BODY FLUID OF ASCARIS LUMBRICOIDES

In nematodes the space between the body wall and the gut wall is filled with a fluid which in the case of such large-sized worms as those of the genus Ascaris is available in quantities sufficient for investigation. According to Flury the body fluid of *Ascaris equorum* consists largely of water (95 per cent). Other substances present in this fluid, according to the same investigator, are albumin, globulin, and other proteins, soaps, free fatty acids, various katabolic products of proteins, purin bases, and their derivatives, sodium chlorid and other inorganic substances, as well as digestive and oxidizing enzyms. Flury found that the body fluid of *A. lumbricoides* is physically and chemically indistinguishable from that of *A. equorum*.

The fact that the body fluid of *Ascaris lumbricoides*, which in fresh specimens has a bright pinkish color, contains oxyhemoglobin is of great significance. The presence of oxyhemoglobin in the worms may be readily demonstrated by means of the spectroscope. Schimmelpfennig (*1902*) appears to have been the first investigator to note this fact, on the basis of which he ascribed to worms of the genus Ascaris the rôle of bloodsuckers. This investigator also states that worms belonging to this genus liberate their oxyhemoglobin content into the physiological salt solution in which they are kept alive in vitro. The presence of iron granules in the gut wall of ascarids was affirmed by Askanazy (*1896*), who bases his view on positive Berlin blue tests, the inference being that the pigment in question is obtained from the blood of the host. Flury (*1912*) refers to the presence of hemoglobin in ascarids and states that he observed it in worms which had been kept for two weeks in an incubator. Flury

inclines to the view that oxyhemoglobin is a normal constituent of these worms. Dobernecker (*1912*) records the presence of oxyhemoglobin in ascarids, which he determined by means of the spectroscope. Fauré-Fremiet (*1913*) expresses the view that the oxyhemoglobin present in the intestine of worms belonging to the genus Ascaris is obtained from the blood of the host and that the iron pigment in the intestinal cells is derived from disintegration products of hemoglobin. Galli-Valerio (*1915*) affirms the presence of blood in ascarids and states that the body fluid of a female ascarid gave a positive benzidin test for blood. The present writer (Schwartz, *1919*) found that *Ascaris lumbricoides* loses its oxyhemoglobin when kept in vitro for a number of days and that coincident with the loss of this substance the worms become sluggish and die. Magath (*1919*) has made a similar observation in the case of another nematode (*Camallanus americanus*) which contains a "reddish fluid." Magath also notes the presence of pigment granules in the gut wall of this worm.

It has already been stated in another section of this paper that Schimmelpfennig (*1902*) and Flury (*1912*) found that the body fluid of worms belonging to the genus Ascaris is destructive to red blood cells. Following are the observations and experiments of the present writer on this question.

Fluid collected from fresh specimens of *Ascaris lumbricoides* within 24 hours after removing the parasites from the host is not hemolytic. Such fluid was tested on the washed erythrocytes of cattle, sheep, hog, rabbit, and guinea pig without producing any appreciable dissolving action. In one case it was found that fluid which had been kept in a refrigerator for three days was destructive to sheep erythrocytes, but a repetition of this experiment with fluid from another lot of worms yielded negative results. Fluid collected under aseptic precautions and kept in a refrigerator for two or three days failed to hemolyze red blood corpuscles.

On the other hand, fluid from worms which had been kept alive in vitro for a number of days was found to be hemolytic. In one case worms were kept alive in a physiological salt solution for eight days at a temperature of 32° to 33° C. At the end of this period fluid was obtained from the worms and tested on the washed red blood cells of the hog, with positive results. A repetition of this experiment on a different sample of washed erythrocytes from the hog likewise yielded positive results. In another case worms which were kept alive for six days yielded a fluid which was destructive to washed sheep corpuscles. Fluid from another lot of worms which had been kept in the laboratory for four days was but slightly although quite unmistakably hemolytic to sheep blood corpuscles. A portion of this fluid was boiled and the clear liquid after being separated from the coagulum was still hemolytic. Fluid from

worms which has been kept alive for eight days was strongly hemolytic to washed sheep blood corpuscles.

In the course of these experiments it was observed that whereas fresh specimens of *Ascaris lumbricoides* from swine are pink in appearance they become white as they are kept in the laboratory. Spectroscopic examination of the fluid showed that the pink appearance is correlated with the presence of oxyhemoglobin and the white appearance is correlated with the absence of that substance. In other words, worms kept in vitro lose their oxyhemoglobin, a fact which appears to indicate that this substance is not a constant constituent of the worm but that it is obtained from the host, the supply evidently being renewed from time to time. Inasmuch as Schimmelpfennig (*1902*) states that the oxyhemoglobin is eliminated in vitro, the present writer made spectroscopic examinations of physiological salt solution in which ascarids had been kept alive for 24 hours or longer, and found that such solutions did not show the oxyhemoglobin spectrum. Tests for iron in such salt solutions showed but slight traces of this substance. That these traces were excretion products of the parasite was shown by the fact that a quantity of salt solution from the same supply which was added to the beakers in which the worms were kept gave negative results. It may be concluded, therefore, that when removed from the host and kept in a physiological salt solution living ascarids lose their oxyhemoglobin content not by excreting it as such but probably by breaking it down into simpler substances and storing the iron in their tissues. The fact that ascarids are rich in iron and that this substance enters in considerable quantities into the composition of the eggs (Schimmelpfennig, *1902*) is decidedly significant in this connection.

On the basis of certain experiments Flury (*1912*) states that salt solutions in which living ascarids have been kept for 24 hours have absorbed the hemolysin which the parasites excrete. The observations of the present writer on this point do not bear out Flury's view, as the following experiments will show.

A number of swine ascarids were kept in a beaker for 24 hours in a quantity of physiological salt solution sufficient to cover the worms. Ten cc. of this salt solution produced no dissolving effect on 1 cc. of a 5 per cent suspension of guinea-pig red blood corpuscles. A similar experiment was performed with a different lot of worms, the salt solution in this case being tested on washed hog erythrocytes, with negative results. Negative results on sheep erythrocytes were also obtained with salt solution in which another lot of worms had been kept for 24 hours. In a similar way negative results were obtained on several other occasions with salt solution in which living ascarids had remained from 18 to 36 hours.

In the experiments mentioned above the parasites were examined and found to be still alive before the salt solution was tested as to its hemolytic

property. In another series of experiments in which some of the worms were found to be dead it was observed that the salt solution in which they had been kept was destructive to red blood corpuscles. That the hemolytic effects of salt solution in which dead ascarids had been kept was independent of bacteria was shown by the fact that the salt solution was free from putrefactive odors associated with decay, due to the precautions which were taken to free the parasites from bacteria by immersing them in 2 per cent formalin and washing them first in water and then in salt solution before subjecting them to these experiments. In one experiment which was conducted under strictly aseptic precautions the worms were thoroughly washed in running water, in formalin, and in sterile salt solution in the order indicated and then placed in sterile flasks containing an 0.85 per cent solution of sodium chlorid. These flasks were placed in an incubator at 37° C. for several days. The worms died but the fluid showed no cloudiness. Transfers of portions of this fluid to culture media (nutrient broth and agar) failed to produce bacterial growth despite the fact that the tubes containing the media were kept in the incubator for a week. The sterile salt solution in which the ascarids died was hemolytic to washed sheep corpuscles.

These facts appear to indicate that Ascaris hemolysin is closely bound to the cells of the parasites and becomes dissociated from them rather easily after death of the worms, a view which is in harmony with the observation of Tallqvist (*1907*) with reference to the hemolysin from *Diphyllobothrium latum*. The fact that the body fluid of worms which have been kept in vitro for a number of days becomes hemolytic is entirely in harmony with that view, since, under conditions of starvation, autolysis of the tissues of the parasites undoubtedly takes place, especially after the storage products, largely glycogen,[1] are consumed.

### 3. EXPERIMENTS ON THE POSSIBLE PRESENCE OF COMPLEMENT IN THE BODY FLUIDS OF ASCARIS LUMBRICOIDES

Experiments with body fluid from fresh specimens of *Ascaris lumbricoides* were performed with a view to determining whether it contains a substance capable of activating a hemolytic system. As is well known, washed red blood corpuscles to which a specific inactivated antiserum is added will not hemolyze unless a certain quantity of normal fresh blood serum is added. The substance in the normal blood serum which in itself has no hemolytic power but which activates inactivated antiserum is known as alexin or complement. Comparatively little is known of this body except that it is a normal constituent of blood serum, that it deteriorates rapidly in vitro, and that it is destroyed by heating at 56° C. for 30 minutes. According to Noguchi (*1907*), soluble soaps to which

[1] Schulte and Krummacher (*1915*) have shown that starving ascarids do not consume their fat content and have confirmed Weinland's views with reference to the rôle of glycogen in the metabolism of the worms in vitro.

inactivated serum is added act as complement; in other words, a mixture of inactivated serum and soap can activate a hemolytic system (washed red blood corpuscles plus specific antiserum).

The present writer endeavored to answer the following questions: Is the fresh body fluid of *Ascaris lumbricoides*, which, as has already been shown, has no hemolytic power, capable of activating a hemolytic system? In other words, does it contain complement? Second, can a combination of inactivated serum and an alcoholic extract of body substance of *A. lumbricoides* from which the ether-soluble fraction has been removed, and which contains whatever soluble soaps the parasite has,[1] activate a hemolytic system? The answers to these questions will be found in the results of the following experiments.

One cc. of washed sheep red blood corpuscles was mixed with a unit of specific inactivated antiserum (amboceptor) determined by previous titration. To one tube containing this mixture a certain quantity of fresh guinea-pig serum (complement) was added, sufficient to activate the amboceptor—that is, to cause it to combine with the blood corpuscles and to produce hemolysis. The quantity of complement necessary to activate the hemolytic system was determined by previous titration. Hemolysis was produced in 30 minutes at 37° C. To a series of 10 tubes containing the mixture of amboceptor and sheep red blood corpuscles various quantities of body fluid collected from living swine ascarids under aseptic precautions shortly after the worms had been removed from their hosts were added. The quantities of fluid added to these tubes ranged from 0.1 cc. to 10 cc. These tubes were shaken and incubated at 37° C. for one hour. No hemolysis was observed in any tube. The tubes were then put in a refrigerator for 20 hours longer, but the blood corpuscles remained intact. It should be stated in this connection that the body fluid in question was free from bacteria, since a portion of it was thoroughly mixed with melted agar which was plated and incubated. The plates remained sterile. Ascaris fluid lacks, therefore, a substance (complement) which is capable of activating a hemolytic system.

As to the combination of inactivated normal serum with an alcoholic extract of *Ascaris lumbricoides*, the following experiment was performed: Dried ascarids were powdered, extracted in warm alcohol, and the alcoholic extract after evaporating the alcohol was washed with ether. The ether, as is known, removes neutral fats, fatty acids, lecithin, cholesterin, and other lipoids. The ether-insoluble substance was then dissolved in salt solution and combined with normal guinea-pig serum that had been heated to 51° C. to determine whether this combination can act as complement, that is, whether it can activate a hemolytic system.[2] To one

[1] The presence of soaps in ascarids is affirmed by Flury (*1912*).

[2] According to Noguchi, similar chemical fractions of mammalian tissues combined with inactivated normal serum act as complement.

tube containing 1 cc. of a mixture of washed sheep red blood corpuscles and specific antiserum in the proper proportion as determined by previous titration, one unit of normal guinea-pig serum (complement) was added. (The unit of complement was determined by titration.) Hemolysis resulted. To a second tube containing a mixture of washed sheep red blood cells and specific antiserum one unit of inactivated complement (heated to 51°) was added. No hemolysis resulted. To a series of tubes containing washed sheep red blood corpuscles and specific antiserum various combinations of inactivated guinea-pig complement and alcoholic extract of *A. lumbricoides* were added. No hemolysis was produced in any of these tubes. It is evident, therefore, that *A. lumbricoides* not only lacks complement but that an alcoholic extract of the worm freed from all ether-soluble substances combined with inactivated normal serum can not act as complement.

In this connection it is of interest to note that Holland (*1919*) found that the blood of insects lacks complement and that this substance is also absent from the blood of mollusks. Cantacuzene (*1919*) examined the fluids of a number of invertebrates as well as of tunicates but failed to find complement. He succeeded, however, in producing complement in a crab (a species of Eupagurus) by artificial immunization with sheep red blood corpuscles.

Summarizing, *Ascaris lumbricoides* in common with other invertebrates lacks complement, a substance that is known to play an important rôle in the immunity processes of higher vertebrates. That *A. lumbricoides* and other internal parasites which live in parts of the body where bacteria are more or less abundant protect themselves against bacterial invasion is probable. The intestine of *A. lumbricoides* contains bacteria, as has been recorded by several investigators. The present writer found bacteria in the intestine, but the body fluid of fresh ascarids when collected under aseptic precautions was found to be sterile. That the body fluid and tissue extracts of ascarids and of other internal parasites contain bactericidal substances has been affirmed by a number of writers (Alessandrini, *1913*).

#### 4. EXPERIMENTS WITH EXTRACTS OF ENTIRE WORMS

It has already been stated that Weinberg (*1907*), Whipple (*1909*), and Alessandrini (*1913*) failed to find hemolysins in salt-solution extracts of ascarids. Garin (*1913*) records the results of 10 experiments with extract of worms of the genus Belascaris, of which 8 yielded negative results and 2 yielded positive results on dog-blood corpuscles. These investigators experimented with extracts of fresh specimens made by macerating the worm material in physiological salt solutions. The present writer found that as a result of extracting *Ascaris lumbricoides* material by macerating fresh worm substance in salt solutions the hemolysin is seldom liberated

from the tissues of worms. Better results were obtained by grinding up fresh worm material with sand and shaking the mixture of worm fragments and sand for a number of hours, followed by extraction in an incubator for a number of days. This procedure necessitated the addition of a preservative to the extract in order to prevent bacterial contamination. In experiments in which this procedure was followed, sufficient carbolic acid was added to make a 0.25 per cent solution; and in hemolytic tests controls involving the use of salt solution containing a similar quantity of carbolic acid were included. Following the procedure described above an extract of fresh worm material was made as follows: A few pieces (10 gm. by weight) of worm material from a number of different specimens were ground up with sand and suspended in 100 cc. of physiological salt solution containing 0.25 per cent of phenol. The mixture was shaken for a few hours in a shaking machine and then incubated, usually for three days, at 37° C. The extract was then filtered and a clear filtrate tested on various samples of red blood corpuscles as follows.

The filtrate was tested on washed erythrocytes of a number of cattle, sheep, hogs, rabbits, guinea pigs, and rats, with positive results. In most experiments it was found that 0.4 cc. of the extract hemolyzed 1 cc. of a 5 per cent suspension of red blood corpuscles. In a number of tests 0.2 cc. of the extract hemolyzed 1 cc. of the suspension of corpuscles. As a control on the phenol which was added as a preservative, 0.5 cc. and 1 cc. of a salt solution containing ½ per cent of phenol was tested on each sample of blood corpuscles used in the hemolytic tests, with negative results. Tests to determine whether normal serum contains antibodies were nearly always positive. From 0.2 to 0.5 cc. of serum was sufficient to inhibit hemolysis of 1 cc. of corpuscle suspension by from 0.2 to 0.4 cc. of the extract. Sometimes 0.1 cc. of serum brought about the same results.

That the activity of the hemolysis is independent of the acidity of the solution was shown by the fact that as a result of neutralizing the extract its activity was not destroyed. Furthermore, the hemolytic potency of the extract was not due to secondary degeneration products associated with acid production, because the hemolytic power of the extracts remained intact for a long period (several months), during which it was tested from time to time against different species of corpuscles. Moreover, filtrates of extracts of worms that were prepared by thoroughly triturating the specimens and adding a few drops of chloroform to inhibit bacterial growth during the few hours that the extracts were kept in a refrigerator were found to be hemolytic. An example of the results of experiments with salt-solution extracts of *Ascaris lumbricoides* on red blood cells is given in Table I, in which a few experiments are summarized.

TABLE I.—*Effect of salt-solution extract of Ascaris lumbricoides on red blood corpuscles* [a]

| Kind of erythrocytes.[b] | Quantity of extract.[c] | Results after two hours at 37° C. |
|---|---|---|
| Guinea pig | 0.1 cc | − |
| Do | .2 cc.[d] | +++ |
| Do | Salt solution | − |
| Do | .1 cc. (boiled) | − |
| Do | .2 cc. (boiled) | +++ |
| Rat [e] | .1 cc | + |
| Do | .2 cc | ++ |
| Do | .3 cc | +++ |
| Do | Salt solution [d] | − |
| Hog [f] | .2 cc | + |
| Do | .4 cc | +++ |
| Do | Salt solution [d] | − |
| | .2 cc | − |
| Cattle [f] | | |
| Do | .4 cc | +++ |
| Do | Salt solution [d] | − |

[a] − indicates total absence of hemolysis. + indicates slight hemolysis. ++ indicates marked but incomplete hemolysis. +++ indicates complete hemolysis.

[b] One cc. of a 5 per cent suspension of defibrinated blood washed three times in physiological salt solution was used in experiments.

[c] The extract used in these experiments was made by suspending 10 gm. of fresh worm material in 100 cc. of 0.85 per cent NaCl.

[d] Two controls—0.5 cc. and 1 cc. of salt solution containing 0.5 per cent phenol were tested on 1 cc. of the suspension of corpuscles.

[e] Pooled blood from six rats.

[f] Four samples of corpuscles were tested.

## 5. EXPERIMENTS WITH ASCARIS LUMBRICOIDES POWDER

The hemolytic principle of *Ascaris lumbricoides* may be preserved by drying the parasites. Specimens collected at a local abattoir were washed in salt solution to remove adhering interstinal débris, dried superficially with filter paper, and then placed in vacuum over sulphuric acid. When the specimens were sufficiently crisp they were powdered in a mortar and stored for future use. *Ascaris lumbricoides* powder when added to a suspension of washed blood cells of cattle, sheep, swine, etc., produces rapid hemolysis. As in the case of extracts of the parasite, the hemolytic action is inhibited by normal serum. The hemolytic substance may be more easily obtained from dried than from fresh ascarids by extracting the worm material in physiological salt solution. This is no doubt due to the fact that the dried material can be readily crushed and the hemolytic substance which, as has already been indicated, is rather closely bound to the parasite, may be more readily liberated. The following experiments performed by the writer illustrate this point: Several swine ascarids were broken up into small fragments but were not powdered in a mortar. A portion of this material was extracted in salt solution for a few hours and filtered. The filtrate was tested on washed sheep corpuscles with negative results. The remaining portion of dried worm material was thoroughly ground

in a mortar, extracted in physiological salt solution, filtered, and the filtrate tested on sheep corpuscles. The results in this case were positive.

A number of experiments were made with salt-solution extracts of powdered *Ascaris lumbricoides*. Rabbit and sheep corpuscles were used in nearly all experiments with these extracts. The results of these experiments were positive when the extracts were made from thoroughly powdered material; otherwise the extracts were only slightly hemolytic.

Extracts of powdered material of *Ascaris lumbricoides* were usually prepared as follows: A definite quantity of powder was added to a definite volume of physiological salt solution in a flask, the latter was shaken thoroughly, and the material was extracted for a few hours to two days in a refrigerator without the addition of any preservative, or extracted in an incubator, in which case a few drops of chloroform were added. The mixtures were then filtered, and in cases in which chloroform had been added the filtrate was left in an open receptacle in order to get rid of the chloroform by evaporation. The salt-solution filtrates were then tested as to their hemolytic power.

An example of results of these experiments is given in Table II, in which a number of tests are summarized.

TABLE II.—*Effects of salt-solution extracts of powdered Ascaris lumbricoides on red blood corpuscles* [a]

| Experiment No. | Kind of erythrocytes.[b] | Quantity of extract.[c] | Results after 2 hours at 37° C. | Results after 20 hours.[d] |
|---|---|---|---|---|
| 1 | Rabbit | 5 drops | − | +++ |
| 2 | ...do | 8 drops | +++ | +++ |
| 3 | ...do | 8 drops (boiled) | − | .......... |
| 4 | ...do | 10 drops (boiled) | ++ | .......... |
| 5 | ...do | 10 drops (heated at 60° C. 30 minutes) | +++ | − |
| 6 | ...do | 10 drops salt solution | − | − |
| 7 | Sheep | 8 drops | − | +++ |
| 8 | ...do | 10 drops | +++ | .......... |
| 9 | ...do | 10 drops salt solution | − | − |
| 10 | ...do | 8 drops | + | .......... |
| 11 | ...do | 10 drops | ++ | .......... |
| 12 | ...do | 10 drops salt solution | − | − |

[a] − indicates negative results. + indicates slight hemolysis. ++ indicates marked but incomplete hemolysis. +++ indicates complete hemolysis.

[b] Five drops of a 5 per cent suspension of washed rabbit erythrocytes and a 3 per cent suspension of washed sheep erythrocytes were used in these experiments.

[c] In experiments 1 to 8, inclusive, the following extract was used: 0.85 gm. of powder were suspended in 85 cc. of salt solution and extracted in an incubator for 24 hours. In experiments 10 to 12 the extraction was made as follows: 1 gm. of powder was extracted in 10 cc. of salt solution in a refrigerator.

[d] After remaining in an incubator for 2 hours the tubes containing the corpuscles and extracts were in some instances transferred to a refrigerator (8° C.) where they were kept for 18 hours longer before the final reading was taken.

## 6. EXPERIMENTS WITH EXTRACTS OF DIFFERENT ORGANS OF ASCARIS LUMBRICOIDES

It has already been stated that the body fluid of fresh specimens of *Ascaris lumbricoides* is not hemolytic and that this fluid acquires hemolytic properties as the parasites are kept in vitro. Extracts of entire worms, on the other hand, were found to contain a hemolytic substance which is apparently firmly bound to the tissues of the parasite. These facts appear to indicate that the hemolytic substance is liberated in rather small quantities and that it ultimately finds its way into the body fluid. That the liberation of hemolysin from the tissues and cells of the parasite is associated with metabolic processes of the worms is advanced as a plausible explanation of the facts. In the host animal the body fluid of the worm contains blood and blood products by which the hemolysin is apparently neutralized. In vitro, on the other hand, the blood elements disappear, as judged by the disappearance of oxyhemoglobin; and meanwhile fresh hemolysin which has found its way into the fluid remains unbound.

The question as to which morphological elements of *Ascaris lumbricoides* secrete the hemolytic substance or substances is interesting. A number of specimens of the parasite were therefore dissected and the intestine, reprodnctive organs, and body wall were separated into different lots. Physiological salt-solution extracts from each lot were tested on hog blood, and in a few cases on sheep blood.

In one series of experiments it was found that the extracts of the intestine were strongly hemolytic, whereas extracts of the body wall showed no hemolytic effects. Extracts of the reproductive organs were moderately hemolytic. In a second series of experiments extracts of the intestine were found to be very markedly hemolytic, whereas extracts of the body wall and reproductive organs showed weak hemolytic power.

In another series of experiments a number of worms were dissected, and the body wall, reproductive organs, and chyle intestine were separated into different lots. Each lot was washed in physiological salt solution and dried with filter paper. The material in each lot was then put in an incubator at 40° C. and allowed to remain there for 24 hours. Pulverized material from each lot was then suspended in physiological salt solution and tested on washed sheep corpuscles. Extract of the intestine produced rapid hemolysis at 37° (in about 1 hour), whereas extract of body wall of approximately the same strength as that of the intestine produced no hemolysis even after 3 hours at 37° followed by 18 hours in a refrigerator. Extract of the reproductive organs produced no hemolysis after 3 hours at 37° but after an additional period of 18 hours at 8° a slight indication of hemolysis was observed.

It may be concluded, therefore, that the hemolytic agent of *Ascaris lumbricoides* is primarily a secretory product of the intestine and that part of this substance finds its way into the body fluid where it is apparently neutralized by blood elements that are obtained from the host.

## 7. EXPERIMENTS WITH DIFFERENT CHEMICAL FRACTIONS OF ASCARIS LUMBRICOIDES

In contrast to the comparatively slight solubility of the hemolytic substance of *Ascaris lumbricoides* in physiological salt solution is its ready solubility in lipoid solvents, especially in alcohol. Equal quantities of powder were suspended in 5 cc. each of physiological salt solution, 95 per cent alcohol, ether, and acetone for 48 hours. The filtrates were evaporated and redissolved in 5 cc. of physiological salt solution. These extracts were then tested on a 5 per cent suspension of washed rabbit red blood cells. The alcoholic extract was the most potent from the point of view of hemolysis. Acetone and ether extracts were about as potent as the physiological salt-solution extract. In a second series of experiments in which *A. lumbricoides* powder was extracted in the substances referred to above, the extracts were tested on sheep red blood cells. In those experiments the alcoholic extract was the most potent, while the physiological salt-solution extract and the ether extract were the least potent.

Further experiments with different fractions of *Ascaris lumbricoides* were performed. Dried worm material was ground up in a mortar and extracted in four volumes of ether in a flask for 48 hours at 37° C. The ether was then removed from the worm material and saved and fresh ether was added to the flask. This was allowed to extract for 24 hours, the ether being removed at the end of that period and added to the first ether extract. To the worm material fresh ether was again added, and after 24 hours of extraction the mixture was filtered. The last ether filtrate was practically free from any extract. A portion of the ether extract was then evaporated and a brownish yellow fatty substance left behind. This substance had the characteristic odor of *A. lumbricoides*. A small quantity of this substance was emulsified in physiological salt solution and tested on washed rabbit blood corpuscles, which it hemolyzed. A second portion of ether extract in solution was shaken with an equal quantity of distilled water and allowed to remain at room temperature for two hours. Two layers—namely, an ether layer (fraction 1) and a water layer (fraction 2)—were separated. The ether layer (fraction 1) was evaporated, and a fatty substance was left behind which was hemolytic to washed sheep corpuscles. A portion of this substance was redissolved in ether, and to this solution an equal quantity of a solution of sodium bicarbonate was added and the mixture was thoroughly shaken. The ether layer (fraction 1*a*) was removed

and evaporated. A fatty substance free from the characteristic odor of *A. lumbricoides* was left after evaporating the ether. This substance had no hemolytic power. Inasmuch as sodium bicarbonate saponified the free fatty acids in the ether, it is evident that the hemolytic effect of the ether extract free from the water-soluble fraction is due to fatty acids. Flury (*1912*), in fact, came to the conclusion that the hemolytic power of ascarids is to be ascribed to free fatty acids of which the unsaturated fatty acids are of prime importance. Flury stated furthermore that oleic acid is probably the most active principle of Ascaris hemolysin because of the known hemolytic powers of this substance. The watery layer (fraction 2) was opalescent and contained a thick suspension of a grayish substance which was found to be slightly hemolytic to sheep cells.

The ether extract contains therefore two fractions, (1) a water-insoluble fraction which consists of neutral fats and fatty acids, and (2) a water-soluble fraction, both of which are hemolytic. The composition of the water-soluble substance was not definitely determined. This substance was tested and found to be soluble in 95 per cent alcohol and in hot and cold water. By acidifying a watery solution of the substance and shaking it with an equal volume of ether it was made to go into solution and was recovered in the ether layer. Another portion of the water-soluble substance was salted out from water by adding a few drops of a strong solution of sodium chlorid. It rose to the surface, where it formed a thick layer which was insoluble in salt solution. Bondouy (*1908*, *1910*), who experimented with a similar chemical fraction of a species of Strongylus, identified it as consisting of soluble soaps, substances that are known to have hemolytic power.

To recapitulate, an ether extract of *Ascaris lumbricoides* was divided into the following fractions: (1) An ether-soluble and water-insoluble fraction, and (2) a water-soluble fraction. Both fractions were hemolytic, the latter, however, only to a moderate degree. The fatty acid in the first fraction (fraction 1) was saponified. The fatty acid-free fraction which was extracted in ether was not hemolytic. This fraction consists largely of neutral fats. The hemolytic potency of the ether extract of *A. lumbricoides* is therefore due largely to free fatty acid. That the water-soluble part of the ether fraction (fraction 2) is a mixture of soaps is probable.

A portion of the remaining *Ascaris lumbricoides* powder (free from ether-soluble fraction) was extracted in distilled water for 48 hours in an incubator. The mixture was then filtered. The filtrate had a brownish color and a sweetish odor. Tests for proteins were positive. The residue was evaporated at 40° C. A portion of the residue was taken up in salt solution, to which it gave a yellowish coloration. Tested for its hemolytic power on sheep blood corpuscles, it produced rapid hemolysis. The remaining portion of the residue was extracted in 95 per cent alcohol for

24 hours. It was only partly soluble. After filtering off the alcohol, fresh alcohol was added and the extraction continued for 24 hours longer. The alcoholic extracts were evaporated and the residue was taken up with a small quantity of physiological salt solution. Tested for its hemolytic power, the results were strongly positive on sheep erythrocytes. The alcohol-insoluble fraction was not hemolytic even when large quantities were employed.

These experiments are rather significant in view of the fact that they show quite conclusively that the hemolytic potency of *Ascaris lumbricoides* extracts are due not to fatty acids alone but that another substance or substances, soluble in alcohol and water, must be involved.

The experiments described above were repeated several months later with similar results.

Extracts of powdered ascarids in 95 per cent alcohol were made by adding about 6 volumes of alcohol to 1 volume of powder and removing the alcohol by filtration at intervals of two to three days and adding fresh alcohol. After evaporating the filtrates, which were all mixed together, a brownish residue was left behind which was only partly soluble in ether. The ether-soluble portion as well as the ether-insoluble portion was hemolytic. A portion of the powder, free from the alcohol-soluble fraction, was extracted in ether, but when the latter was removed and evaporated no residue was left behind. The remaining portion of the powder free from the alcohol-soluble portion was extracted in physiological salt solution, and this extract when tested on red blood cells was found to be nonhemolytic. These experiments show, therefore, that the hemolytic substances of *Ascaris lumbricoides* are all soluble in alcohol, and confirm the results of the earlier series of experiments with reference to the fact that the ether-soluble fraction of *A. lumbricoides* contains but a portion of the hemolytic substance.

Part of the alcoholic extract was divided into two fractions—namely, an absolute alcohol-soluble fraction and an absolute alcohol-insoluble fraction. The latter was hemolytic, whereas the former showed no hemolytic power.

An ether extract of *Ascaris lumbricoides* powder was redissolved in ether and divided into two fractions by adding acetone in excess, which resulted in the formation of a whitish precipitate. The precipitate was separated from the solution and found to be nonhemolytic. The acetone-ether solution was evaporated and taken up in salt solution. It was also found to be nonhemolytic, whereas prior to precipitation with acetone the ether extract was hemolytic. The precipitate was obtained in quantities insufficient to determine its nature. That it was probably largely lecithin [1] can hardly be doubted. As is known, lecithin in quantities in which it alone produces no hemolytic effect can activate other substances and cause them to produce hemolysis. That this actually occurs in the

[1] The presence of lecithin in ascarids was demonstrated by Flury (*1912*).

case of the ether-soluble hemolytic substance of *A. lumbricoides* appears probable from the experiments described above.

It should also be stated that a 95 per cent alcohol extract of *Ascaris lumbricoides* developed a precipitate when kept in solution in 95 per cent alcohol at 8° C. This precipitate went into solution when the alcohol containing the extract was transferred to room temperature. The removal of this precipitate by filtering in a refrigerator yielded a whitish substance which had no hemolytic power, nor did the removal of this substance interfere with the hemolytic potency of the extract.

8. PROPERTIES OF ASCARIS LUMBRICOIDES HEMOLYSIN

At low temperatures ranging from 6° to 10° C. hemolytic extracts of *Ascaris lumbricoides* lose their potency. Mixtures of extracts and susceptible corpuscles that showed complete hemolysis after 2 hours' incubation at 37° showed no trace of hemolysis after 24 hours at 8°. After being removed from the low temperatures and transferred to an incubator hemolysis occurred rapidly in such mixtures.

In order to determine whether the hemolytic substance of *Ascaris lumbricoides* is absorbed by the red blood cells at low temperatures the following experiments were performed.

Mixtures of washed red blood cells (rabbit and sheep) and hemolytic extracts were put in a refrigerator at 8° C. After 24 hours the supernatant fluid was removed from the corpuscles and the latter were washed three times in succession to free them from traces of extracts; to the washed corpuscles from which the supernatant fluid had been removed an equal quantity of salt solution was added, and the tubes were thoroughly shaken and placed in the incubator. Hemolysis set in slowly. The supernatant fluid which was removed from the corpuscles was also tested as to its hemolytic potency, with inconstant results. In some cases it was found that it had lost its hemolytic potency completely, but in a number of cases it still retained its blood-destroying power. That the potency of the fluid that had been in contact with susceptible corpuscles had been considerably reduced was evident, since it had but slight hemolytic power as compared with that of intact extract. Whether the hemolytic substance in contact with susceptible corpuscles at a low temperature becomes fixed to the cells or whether it is precipitated at a low temperature and escapes removal despite repeated washing has not been determined.

Hemolytic extracts of *Ascaris lumbricoides* are highly resistant to heat. Heating at temperatures ranging from 56° to 60° C. for 30 minutes did not weaken the potency of the extracts. An exposure to 70° for two hours failed to destroy the hemolytic substance. Salt-solution extract as well as alcoholic extracts were heated to boiling, and after cooling they were tested on susceptible red blood cells. It was found that as a

result of boiling the potency of the extracts was weakened but not destroyed.

The hemolysin goes through the pores of Berkefeld, Chamberland, and diatomaceous filters. The filtrates are less potent, however, than nonfiltered solutions.

## V. EXPERIMENTS WITH AGGLUTINATING SUBSTANCES FROM ASCARIS LUMBRICOIDES

In the course of experiments on hemolysis of red blood cells by extracts of *Ascaris lumbricoides* it was observed that the cells frequently became agglutinated before hemolysis set in. The agglutinating effect of the extracts was especially marked on rabbit red blood cells and was observed only occasionally on sheep erythrocytes. Several experiments on hog erythrocytes showed them to be refractory to the agglutinating substance of the parasite.

The agglutinating property of *Ascaris lumbricoides* with respect to rabbit-blood corpuscles was present almost invariably in physiological salt-solution extracts. Alcohol and ether extracts of entire worms were not entirely free from agglutinating properties, however. Unlike the hemolytic substances which are entirely removed from the worm material by alcohol and ether extraction, the agglutinating substance resists extraction in these solvents and may be recovered in the fraction of the worm material from which the alcohol-soluble and ether-soluble fractions have been removed. The salt-solution-soluble hemagglutinin does not appear as firmly bound to the cells of the parasites as the lipoidal hemolysin. The latter, as has already been stated elsewhere in this paper, is but slightly soluble in physiological salt solution unless the material is thoroughly triturated. Salt-solution extracts of coarsely powdered worm material that yield but a small quantity of hemolysin were found to contain a considerable quantity of agglutinating substance. In physiological salt-solution extracts of *Ascaris lumbricoides* that contain the hemolysin and the hemagglutinin the potency of the former may be suppressed by low temperatures (6° to 10° C.), whereas that of the latter remains unaffected by those temperatures.

The hemagglutinin from *Ascaris lumbricoides* is relatively thermostabile and differs in this respect from the hemagglutinin which Tallqvist (*1907*) isolated from *Diphyllobothrium latum*. The latter is injured by 30 minutes' heating at 55° C., whereas that of *A. lumbricoides* withstands heating at temperatures ranging from 56° to 60° for 30 minutes. Hemagglutinating extract of *A. lumbricoides* was passed through a Chamberland filter without injuring its potency.

Summarizing, it may be stated that in contrast to the lipoidal hemolysin, which is inactive at 6° to 10° C. and which is but slightly soluble in physiological salt solution, the agglutinin of *Ascaris lumbricoides* is readily soluble in salt solution, slightly soluble in ether and alcohol, and

active at low temperatures. It also differs from the hemolysin in its relative specificity for certain species of erythrocytes.

## VI. THE EFFECT OF ASCARIS LUMBRICOIDES FLUID ON COAGULATION OF BLOOD

As has already been stated, Weil and Boyé (*1910*) found that as a result of injecting the fluid of *Ascaris equorum* into rabbits the coagulation of the blood was retarded by 20 minutes. These investigators state, however, that they obtained negative results with rabbit blood in vitro. Leroy (*1910*) likewise observed that the blood of dogs which had received injections of the body fluid of *A. equorum* exhibited a delayed coagulation time. Flury (*1912*) made observations on the coagulation of dog blood in contact with the fluid of ascarids in vitro and records a decided delay. His experiments with human blood were likewise positive.

In view of the contention of Weil and Boyé with reference to rabbit blood in contact with *Ascaris equorum* fluid in vitro, the writer tested freshly drawn rabbit blood to which various quantities of *A. lumbricoides* fluid were added, in order to determine if the coagulation power would be affected. The addition of 3 to 5 drops of the fluid to 10 drops of blood delayed the coagulation time about 15 minutes as compared with that of normal blood. The addition of 8 drops of fluid to 10 drops of blood produced a 35-minute delay, whereas the addition of 10 drops of fluid to an equal quantity of blood resulted in a delay of 42 minutes.

The body fluid of *Ascaris lumbricoides* retards the coagulation of blood in vitro as well as in vivo, but its power in this respect is rather limited.

## VII. EXPERIMENTS WITH HOOKWORM HEMOLYSIN (ANCYLOSTOMA CANINUM)

The anemia which occurs in cases of infestation with hookworms has been ascribed to several different factors. The direct abstraction of blood by the parasites, the possible absorption of toxic substances from the digestive tract as a result of the ulceration of the mucosa, hemorrhages following the laceration of the mucosa by the worms (Loeb and his collaborators), and the absorption by the host of hemolysins secreted by the parasites have been advanced as explanations. The last view was accepted as a plausible explanation before any experimental evidence in favor of it had been advanced. That the data with reference to the production of hemolysins by hookworms appear to show that such absorption probably occurs has already been pointed out elsewhere in this paper.

## 1. EFFECTS OF SALT-SOLUTION EXTRACT OF FRESH WORMS ON RED BLOOD CORPUSCLES

In the following experiments the hemolysin was obtained from about 100 specimens of *Ancylostoma caninum* collected from three dogs. The parasites were put into a bottle containing a physiological salt solution and kept in an ice box for about 24 hours after removal from the hosts, without any apparent loss of vitality.

The extract designated as extract of fresh worms was prepared as follows: The parasites were ground up in a mortar containing a small quantity of a physiological salt solution, and the macerated material was then suspended in about 20 cc. of salt solution, shaken vigorously for a few minutes, and placed in a refrigerator overnight. The supernatant fluid was found to be hemolytic, as the following experiments will show.

EXPERIMENT 1.—To each of three tubes containing 0.5 cc. of a 2 per cent suspension of washed dog erythrocytes there were added, respectively, 5, 8, and 10 drops of the extract of fresh worms. As a control, to a fourth tube containing the same quantity of suspension of corpuscles there were added 10 drops of a salt solution. The tubes were shaken thoroughly and placed in the incubator at a temperature of 37° C. At the end of 30 minutes the tube containing 10 drops of the extract showed complete hemolysis. The tube containing 8 drops of extract showed complete hemolysis 15 minutes later, while the tube containing 5 drops of extract showed partial hemolysis at the end of an hour. The control tube showed no hemolysis. The tubes were kept in a refrigerator overnight and no further change was noted.

EXPERIMENT 2.—To three tubes each containing 10 drops of a 5 per cent suspension of washed sheep corpuscles there were added, respectively, 5, 8, and 10 drops of the extract of fresh worms. It was necessary to incubate the tubes at 37° C. for two hours before hemolysis was produced in any tube. The tube containing 10 drops of extract showed complete hemolysis; the tube containing 8 drops of extract showed partial hemolysis, while the tube containing 5 drops of extract showed no hemolysis. A fourth tube containing 10 drops of corpuscle suspension and 10 drops of salt solution showed no hemolysis. These tubes were kept in a refrigerator overnight with practically no change in results except that hemolysis was complete in the tube containing 8 drops of extract and was faintly indicated in the tube containing 5 drops of extract.

EXPERIMENT 3.—The extract of fresh worms was tested against washed rabbit corpuscles as in experiments 1 and 2. Ten drops of a 3 per cent suspension of washed corpuscles were completely hemolyzed by 5 drops of extract in 20 minutes at a temperature of 37° C. This experiment was controlled as usual.

Experiment 4.—Twelve drops of extract of fresh worms were heated for 30 minutes at a temperature ranging from 56° to 58° C. The addition of 0.5 cc. of washed dog corpuscles from the same lot as that used in experiment 1 resulted in partial hemolysis after one hour of incubation at 37°. The tube was kept in a refrigerator overnight and showed almost complete hemolysis the next day.

Experiment 5.—A quantity of extract of fresh worms was heated at 60° to 65° C. for 50 minutes. To two tubes each containing 10 drops of extract that had been thus heated there were added 5 drops of rabbit and sheep corpuscles, respectively, of the same concentration as noted in experiments 2 and 3. No hemolysis was produced after two hours' incubation at 37°. The tubes were kept in a refrigerator overnight and showed slight hemolysis the following day.

Experiment 6.—Twelve drops of extract of fresh worms were heated to boiling, and after cooling they were added to 0.5 cc. of suspension of dog corpuscles of the same concentration as in experiment 1 and were incubated for one hour, but no hemolysis was produced. After remaining in an ice box overnight the tube showed but a trace of hemolysis. Similar results were obtained when rabbit and sheep corpuscles were used. Control tubes showed no hemolysis.

A second series of experiments with extracts of fresh worms was performed several weeks later. The details of these experiments follow.

The extract referred to as extract II of fresh worms was prepared by macerating 29 live specimens of *Ancylostoma caninum* [1] obtained from five dogs shortly after the animals had been killed. The macerated material was suspended in 3 cc. of physiological salt solution, shaken vigorously, and allowed to extract at room temperature for about an hour before it was tested for its hemolytic power. Part of the extract was kept overnight in a refrigerator and was used the following day. The suspension of corpuscles and extract was incubated at 37° C. for periods shown in the table, the results were noted, and the tubes were then placed in a refrigerator for an additional period of 18 hours, when the final results were read.

The data presented in Table III show that rabbit and dog corpuscles are more susceptible to hookworm hemolysin than the corpuscles of swine and cattle. Despite the fact that the latter were not hemolyzed by the extract used in these tests, they are not absolutely resistant to extracts of dog hookworms, as will be shown in another section of this paper.

[1] These specimens were washed several times in physiological salt solution.

Table III gives a record of the experiments performed with this extract.

TABLE III.—*Effect of extract II of fresh worms (Ancylostoma caninum) on washed red blood corpuscles* [a]

| Kind of corpuscles.[b] | Quantity of extract. | Period of incubation. | Results at end of incubation period. | Results after 18 hours additional in refrigerator (8° C.). |
|---|---|---|---|---|
| Rabbit | 5 drops | 1 hour | +++ | |
| Do | 8 drops | do | +++ | |
| Do | Control [c] | do | — | — |
| Dog | 5 drops | 2¼ hours | — | ++ |
| Do | 8 drops | do | ++ | +++ |
| Do | Control [c] | do | — | — |
| Hog | 5 drops | do | — | +++ |
| Do | 8 drops | do | — | +++ |
| Do | Control [c] | do | — | — |
| Cattle | 5 drops | do | — | — |
| Do | 8 drops | do | — | — |
| Do | Control [c] | do | — | — |

[a] ++ indicates marked though incomplete hemolysis. +++ indicates complete hemolysis. — indicates absence of hemolysis.
[b] 0.2 cc. of a 5 per cent suspension of washed blood corpuscles were used in all experiments summarized in this table.
[c] Eight drops of physiological salt solution were added to the washed blood corpuscles in order to control the experiment.

The sediment in the tube containing the extract of hookworms when shaken with 3 cc. of physiological salt solution yielded additional hemolysis, as the following experiments will show.

EXPERIMENT 7.—After the supernatant fluid from the extract (extract II of fresh worms) had been removed the sediment was shaken up with about 3 cc. of physiological salt solution, which was tested against a 5 per cent suspension of washed dog corpuscles from the same lot as that referred to in Table I. Three drops of corpuscles were completely hemolyzed by three drops of the extract after one hour's incubation at 37° C. This experiment was controlled as usual.

EXPERIMENT 8.—Five drops of the same extract were boiled for about one minute. After cooling, three drops of dog erythrocytes from the same lot as that used in experiment 7 were added and the mixture incubated for 1½ hours at 37° C. No hemolysis was produced. The tube was kept 18 hours in the refrigerator without any change.

## EXPERIMENTS WITH EXTRACTS OF DRIED WORMS

The experiments recorded below were performed with the following extract:

Fifty-eight mgm. of coarsely powdered worm material (*Ancylostoma caninum*) dried at 37° C. and kept in a small vial for about two years were extracted in 10 cc. of physiological salt solution for several hours. Unlike

the extract of fresh worms, which is opalescent, the extract of powdered material remained quite clear.

EXPERIMENT 9.—To four tubes labeled from 1 to 4, each containing 5 drops of a 5 per cent suspension of washed rabbit corpuscles, there were added, respectively, 3, 5, 8, and 10 drops of the extract. To a fifth tube containing an equal quantity of corpuscles there were added 10 drops of physiological salt solution in order to control the results of the experiment. The tubes were incubated for 1 hour at 37° C., and kept for 18 hours longer in a refrigerator, after which the final results were read. Tube 1 showed no hemolysis, while tubes 2, 3, and 4 showed complete hemolysis. The control tube showed no hemolysis.

Additional experiments with the same extract and the same corpuscles showed that the hemolytic action was very slow, since 10 drops of the extract in contact with 5 drops of the suspension of corpuscles failed to produce hemolysis after 2 hours' incubation at 37° C., but after an additional period of 18 hours in a refrigerator the tube showed complete hemolysis, whereas the control tube showed no trace of hemolysis.

EXPERIMENT 10.—The extract of dried worms was tested on a 5 per cent suspension of washed corpuscles of cattle and swine as follows: To four tubes each containing 0.2 cc. of corpuscles there were added, respectively, 1, 2, 3, and 5 drops of the extract, and the tubes were incubated for 1 hour. None of the tubes showed hemolysis. After remaining in a refrigerator overnight the following results were noted.

Cattle corpuscles: The tubes containing 1 and 2 drops of the extract showed partial hemolysis, whereas the tubes containing 3 and 5 drops of extract showed complete hemolysis.

Hog corpuscles: No hemolysis was observed in any tube.

The foregoing experiments were controlled as usual.

In the experiments described above the extract was not filtered but was added to the suspension of corpuscles together with some particles of worm material.

In a second series of experiments performed several weeks later it was found that washed rabbit blood corpuscles were unaffected when placed in contact with an extract of dried hookworms, incubated for 3 hours, and then kept in a refrigerator for an additional period of 18 hours. While no record was made as regards the introduction of particles of worm material into the tubes containing the suspension of corpuscles, it is probable that the clear supernatant fluid alone was added.

A repetition of the experiment on a later date yielded the following results.

EXPERIMENT 11.—A small quantity of coarsely powdered worm material was extracted in physiological salt solution, filtered, and the filtrate tested on washed rabbit blood corpuscles. No hemolysis was produced. To the material which had thus been extracted a small quantity of physiological salt solution was added, the contents were thoroughly agitated,

and a few drops containing worm particles were added to 0.5 cc. of a 5 per cent suspension of washed rabbit erythrocytes. After one hour's incubation hemolysis was complete. A tube containing corpuscle suspension alone showed no hemolysis. A repetition of this experiment yielded similar results.

From the foregoing experiments it appears that the hookworm hemolysin is firmly bound to the cells of the parasite. In fresh worms a considerable quantity of free hemolysin is probably present in the tissues and fluids of the body, which is absorbed by the salt solution in the course of extraction. Since the sediment of extracts of fresh worms has been found to yield additional hemolysin after the first extraction, it is evident that salt solution does not absorb all the hemolysin present in the worms. The observation of Preti (*1908*) that tryptic digestion liberates the hemolysin is further evidence of a close union between the hemolysin and the cells of the worm.

### 3. EXPERIMENTS WITH EXTRACTS OF ALCOHOLIC SPECIMENS

The experiments described below were performed with extracts obtained from specimens of *Ancylostoma caninum* which had been preserved in alcohol for about three years. Unless otherwise stated the extracts were prepared as follows: The specimens were washed several times in distilled water, dried at room temperature, and powdered in a mortar; 0.1 gm. of the powder was suspended in 10 cc. of an 0.85 per cent solution of sodium chlorid and extracted in a refrigerator for about 24 hours. The supernatant fluid was then tested on the washed erythrocytes of rabbit and sheep as follows.

EXPERIMENT 12.—Five drops of a 5 per cent suspension of rabbit corpuscles plus 3 drops of extract showed complete hemolysis at a temperature of 37° C. in 2 hours. Equal parts of extract and corpuscle suspension showed complete hemolysis in 1½ hours. This experiment was controlled as usual.

EXPERIMENT 13.—Five drops of a 5 per cent suspension of washed sheep corpuscles were mixed with 10 drops of extract and incubated for 2 hours without producing any hemolysis. A similar experiment was performed a few months later with negative results, despite the fact that after incubating the mixtures of corpuscles and extract for 2 hours they were kept in a refrigerator for 18 hours longer.

EXPERIMENT 14.—Five drops of a 5 per cent suspension of rabbit corpuscles were not hemolyzed by 5 drops of extract.

EXPERIMENT 15.—A 5 per cent suspension of washed guinea-pig corpuscles resisted hemolysis after remaining in contact for 3 hours at a temperature of 37° C. with an extract of alcoholic specimens made by extracting 200 dried specimens in 6 cc. of physiological salt solution and mixing 3 drops of extract with 2 drops of the suspension of corpuscles. Fifteen drops of the extract in contact with 3 drops of the blood suspen-

sion for 2 hours at 37° C. followed by 48 hours in a refrigerator resulted in partial hemolysis. Several controls in which the suspension of corpuscles alone and equal quantities of the suspension of corpuscles and extract were employed showed complete absence of hemolysis.

These experiments indicate that alcoholic specimens are much less potent in their hemolytic action than fresh specimens. This is doubtless due to the loss of hemolytic substance to the alcohol. In confirmation of this view the writer found that dried hookworms from the dog freed from their ether-soluble and alcohol-soluble fractions were not hemolytic to washed erythrocytes of rabbits. The ether-soluble fraction left rabbit corpuscles intact. The alcoholic extract was unfortunately lost before it was tested for its hemolytic potency.

4. EFFECT OF NORMAL SERUM ON HOOKWORM HEMOLYSIN

EXPERIMENT 16.—To each of four tubes containing 0.5 cc. of blood corpuscles from the same lot as that used in experiment 1 there were added 5 drops of fresh hookworm hemolysin described elsewhere in this paper, and 1, 2, 3, and 5 drops of dog serum, respectively. The tubes were incubated for 1 hour at 37° C. No hemolysis was observed in any of the tubes. After the tubes had remained in an ice box overnight it was found that with the exception of the tube to which but 1 drop of serum was added and which showed a faint trace of hemolysis, inhibition of hemolysis was complete.

EXPERIMENT 17.—Five drops of a 5 per cent suspension of washed rabbit corpuscles from a lot which was susceptible to extract of alcoholic specimens were only partially hemolyzed when 3 drops of normal rabbit serum were added. It was also found that as a result of heating the serum for 30 minutes at a temperature of 56° C. the antihemolytic property was neither destroyed nor impaired.

EXPERIMENT 18.—Washed rabbit corpuscles, which were completely hemolyzed when equal parts of a 5 per cent suspension of cells and equal parts of fresh salt-solution extract were mixed and incubated for 20 minutes at 37° C., were found to resist a double quantity of the hemolysin in the presence of various inactivated sera, as follows: In each of three tubes there were placed 5 drops of the suspension of corpuscles, 10 drops of the extract, and 2 drops of heated rabbit, horse, or dog serum (60° to 65° for 30 minutes). The mixtures were incubated for 2 hours without any resultant injury to the blood corpuscles. After having been kept in a refrigerator for 18 hours after incubation, the tubes containing dog and rabbit serum showed faint traces of hemolysis, while the tube containing horse serum showed no hemolysis.

EXPERIMENT 19.—To each of two tubes containing 3 drops of unwashed rabbit blood there were added 7 drops of physiological salt solution. These mixtures were incubated for 2 hours with 5 and 10 drops of fresh extract, respectively, at 37° C. No hemolysis was produced. The

tubes were kept 18 hours longer in a refrigerator, with a resultant faint indication of hemolysis. Washed erythrocytes from the same rabbit were highly susceptible to the extract, since 10 drops of a 3 per cent suspension of corpuscles were completely hemolyzed by 5 drops of extract in about 20 minutes.

EXPERIMENT 20.—To a series of tubes each containing 3 drops of a 5 per cent suspension of washed dog erythrocytes used in an earlier experiment and included in Table I there were added 5 drops of extract II of fresh worms and various blood sera diluted with an equal quantity of physiological salt solution and heated at 59° C. for 30 minutes. The data and results of these experiments, including the controls, are given in Table IV.

TABLE IV.—*Effects of various sera on hookworm hemolysin* [a]

| Tube No.[b] | Kind and quantity of diluted sera. | Results after 3 hours' incubation at 37° C. | Results after 18 hours longer in refrigerator. |
|---|---|---|---|
| 1 | 3 drops (horse serum) | − | − |
| 2 | 3 drops (dog serum) | − | − |
| 3 | 3 drops (rabbit serum) | − | + |
| 4 | No serum | +++ | +++ |

[a] +++ indicates complete hemolysis. + indicates slight hemolysis. − indicates absence of hemolysis.
[b] Three drops of a 2 per cent suspension of washed dog corpuscles and 5 drops of extract II of fresh worms were used in this series of experiments.

## 5. EFFECT OF COLD ON HOOKWORM HEMOLYSIN

EXPERIMENT 21.—Dog corpuscles which were found to be highly susceptible to an extract of fresh worms at 37° C. remained intact after being kept for 5 hours on ice in contact with a quantity of extract sufficient to destroy the corpuscles at 37° in 30 minutes. The removal of the supernatant fluid following rapid centrifugation showed that it had completely lost its hemolytic potency, since it failed to hemolyze susceptible dog corpuscles after remaining in contact with them for 2 hours at a temperature of 37° followed by 18 hours at a temperature of about 10°

EXPERIMENT 22.—The foregoing experiment was repeated, substituting susceptible rabbit corpuscles for dog corpuscles, with similar results.

The loss of the hemolytic property of the extract in contact with susceptible corpuscles at a low temperature can not be attributed to a possible injurious effect of cold, since it was found that the hemolytic potency of the extract was not injured after standing directly on the ice for 18 hours. Washed sheep corpuscles were readily hemolyzed by the refrigerated extract, whereas a control tube containing corpuscles alone showed no hemolysis.

EXPERIMENT 23.—Six drops of dog blood corpuscles from the same lot as that described in experiment 7 were mixed with 10 drops of extract II

of fresh worms and placed on ice for 3½ hours. The mixture was centrifuged and the supernatant fluid was removed and to it there were added 2 drops of washed dog corpuscles from the same lot as used in the first part of the experiment. After 1 hour's incubation followed by 18 hours in a refrigerator the corpuscles remained intact. The corpuscles from which the supernatant fluid was originally removed were washed three times in salt solution and then incubated with a small quantity of salt solution for 1 hour. Complete hemolysis was produced. A control tube containing a similar quantity of corpuscles without any hemolytic extract showed no hemolysis when placed in an incubator. While this experiment appears to indicate that the hemolysin was fixed to corpuscles and was not removed by repeated washing, this conclusion must be accepted with caution, because the possibility remains that some fragments of worms which were introduced into the tube together with the hemolysin may have been responsible for the hemolysis of the corpuscles after the removal of the supernatant fluid. The fact that the latter had lost its hemolytic power affords, however, strong presumptive evidence of an absorption of the hemolysin by the blood corpuscles.

## 6. DISCUSSION

The results of experiments with reference to the presence of a soluble hemolysin in hookworms (Necator and Ancylostoma) show quite conclusively that when living specimens are macerated in physiological salt solution they yield a considerable quantity of hemolysin. The latter is characterized by relative thermolability, nonspecificity, and susceptibility to normal serum, in the presence of which it loses its potency. So far as its physiological properties are concerned, hookworm hemolysin resembles streptocolysin, staphylolysin, tetanolysin, and other hemolysins of bacterial origin. It differs from the hemolytic substances of *Diphyllobothrium latum* in that it is destroyed by boiling. The conclusion of Preti (*1908*) that hookworm hemolysin is resistant to boiling is not sustained by Whipple (*1909*) and is also contradicted by the results of the present writer's experiments. Unfortunately, Preti has not published a full account of his experiments. His general conclusions are unsupported by details, and judging from the statements that he makes it does not appear that he controlled his experiments.

The present writer's experiments indicate that the hookworm hemolysin is rather firmly bound to the tissues of the parasites, which probably accounts for the difficulty of obtaining strong hemolytic filtrates from salt solution extracts of powdered specimens. That the living worm secretes the hemolysin is evident, however, from experiments with extracts of fresh worms. The unbound hemolysin from fresh specimens evidently disappears in the course of drying. This comparative insolubility of the hemolytic substance from dried specimens in physiological salt solution is perhaps the basis of the contention of Preti (*1908*) and of

Usami and Mano (*1918*) concerning the insolubility in water of the hookworm hemolysin. That Loeb and his collaborators used dried material has already been stated.

The fact that normal blood serum has antilytic properties and inhibits the action of the hookworm hemolysin accounts for the negative results obtained by Loeb and Smith (*1904*) and for the weakly positive results obtained by Whipple (*1909*). In this connection it is important to recall the observations of Noc (*1908*) with reference to the presence of antihemolysins in the blood serum of normal persons and of those recovering from hookworm disease and from beriberi, and the absence of antihemolysins in patients suffering from these diseases. Noc's observations are decidedly significant and do not bear out Whipple's view that the hookworm hemolysin probably bears no relation to the secondary anemia of ancylostomiasis. De Blasi's observations with reference to the presence of hemolysins in the blood serum of patients infected with hookworms and Noc's discovery that under certain conditions the antilytic action of the blood serum may become impaired appear to indicate that the hookworm hemolysin has potentialities of causing anemia and that in severe infections it probably plays an important rôle in the disease.

Since cold (6° to 8° C.) inhibits the action of the hookworm hemolysin in vitro, and the supernatant fluid from tubes in which susceptible blood corpuscles and potent hookworm extract have been in contact for a number of hours at a low temperature no longer has hemolytic properties, the view that the hemolysin is a complex organic substance, not unlike a toxin, in that it apparently consists of haptophore and toxophore groups, appears to be justified. By means of the haptophore group union between the hemolysins and blood corpuscles takes place, but the dissolving or lytic action is produced by the toxophore group. Inasmuch as low temperatures do not appear to interfere with the absorption of the hemolysin by the corpuscles despite the fact that the latter remain undissolved, it is permissible to believe that the toxophore and haptophore groups of the hookworm hemolysin act independently of each other. This view is purely speculative, however, and further experimentation is required before it may be accepted without reservation.

## VIII. EXPERIMENTS WITH EXTRACTS OF CATTLE HOOKWORMS (BUSTOMUM PHLEBOTOMUM)

Hookworms belonging to the genus Bustomum occur as parasites in the small intestine of ruminants. *Bustomum phlebotomum* is the species that infests cattle. According to observations of several investigators, cattle infested with hookworms show symptoms not unlike those of human beings that harbor species of Ancylostoma or Necator.

Experiments with extracts of *Bustomum phlebotomum* similar to those performed with extracts of *Ancylostoma caninum* showed that the former, like the latter, contain a powerful hemolytic agent. The extracts

referred to below were prepared as follows: Living worms were removed from the intestine of a calf, washed a number of times in physiological salt solution, and kept in a refrigerator at a temperature of 8° C. overnight. The following day the worms which were still alive were transferred to fresh salt solution and crushed in a mortar. The crushed material was then suspended in about two volumes of physiological salt solution, shaken thoroughly, and centrifuged. The supernatant fluid which was opalescent was removed and tested as to its hemolytic power. Tested on a 3 per cent suspension of washed sheep blood corpuscles, it was found that 5 drops of the extract hemolyzed 5 drops of the suspension of blood corpuscles in 1 hour at a temperature of 37°. Even 1 drop of extract hemolyzed 5 drops of the suspension of corpuscles after a few hours. Controls, that is, 5 drops of suspension of corpuscles plus 5 drops of salt solution, remained intact. It was observed that before hemolysis set in the contents of the tubes assumed a dark red hue.

An extract from another lot of *Bustomum phlebotomum* prepared as has already been described was tested on a 5 per cent suspension of washed rabbit cells. Five drops of extract produced hemolysis rather slowly upon 5 drops of suspension of corpuscles.

In a third experiment an extract prepared from worms that had been kept in a refrigerator overnight was tested on four different tubes of cattle erythrocytes and on four different tubes of hog erythrocytes. The extract in question was prepared from living specimens as follows: Forty-five specimens were ground up in a mortar and suspended in 2 cc. of physiological salt solution. The suspension was centrifuged, and the opalescent fluid was removed and tested on a 5 per cent suspension of washed blood corpuscles at 37° C. Three drops of extract were added to 5 drops of corpuscle suspension. The experiments with each sample of corpuscle suspension were controlled by adding 3 drops of physiological salt solution to 5 drops of suspension of blood cells. The results of these experiments follow.

CATTLE BLOOD CORPUSCLES.—After 1 hour one tube of blood was partially hemolyzed and three tubes were intact. After 2½ hours two tubes were completely hemolyzed and two were intact. After 3 hours three tubes were hemolyzed; one was intact. The tubes containing the mixtures were placed in a refrigerator at 8° C. until the next day. When examined hemolysis was complete in all tubes. The controls showed no hemolysis.

HOG BLOOD CORPUSCLES.—After 1 hour all tubes were intact. After 1½ hours two tubes were partially hemolyzed and two intact. After 2½ hours two tubes were partially hemolyzed and two completely hemolyzed. After 3 hours all tubes showed complete hemolysis. Controls were intact.

Inasmuch as it was found that the hemolysin could be preserved by drying the worms, powdering the dried material, and storing it in a dark place, further experiments with *Bustomum phlebotomum* hemolysin were performed with dried material. The details of these experiments follow.

To each of four tubes of defibrinated blood (3 drops of physiological salt solution plus 2 drops of blood) a small quantity of the powder was added, and the tubes were shaken thoroughly and placed in an incubator at 37° C. After 2 hours hemolysis was produced in all tubes. Two lots of cattle blood from different animals were collected in a 2 per cent solution of sodium citrate (about 2 volumes of blood to 1 volume of a 2 per cent sodium citrate). Tested against dry *Bustomum phlebotomum* powder the unwashed citrated blood became hemolyzed in about 2 hours at 37°.

Small quantities of powder were also tested on each of four lots of washed cattle blood corpuscles with positive results. Hemolysis set in rapidly and was complete after 1 hour at 37° C.

A few drops of a 3 per cent suspension of washed sheep corpuscles were hemolyzed by a small quantity of *Bustomum phlebotomum* powder. Similar results were obtained with washed rabbit erythrocytes.

*Bustomum phlebotomum* powder extracted in physiological salt solution yields but a small quantity of hemolysin, as the following experiments will show.

Eighty-five mgm. of powder were suspended in 5 cc. of physiological salt solution. A few drops of chloroform were added as a preservative. The mixture was kept at a temperature of 35° to 37° C. for 2 days and then filtered. The clear filtrate was tested on a 5 per cent suspension of washed rabbit cells. Equal parts of filtrate and suspension of cells yielded negative results. It was necessary to add 10 drops of filtrate to 3 drops of corpuscle suspension to produce hemolysis. Evidently the hemolysin is firmly bound to the parasite material and is but slightly soluble in salt solution. In fact, the powder which had been extracted was dried and retested on rabbit blood cells, which it hemolyzed rapidly.

An alcoholic extract of fresh specimens of *Bustomum phlebotomum* was found to be decidedly hemolytic. The extract was prepared as follows: About 100 specimens were washed a number of times in physiological salt solution after they had been removed from the host. The specimens were then triturated in a mortar and extracted in about 2 volumes of 95 per cent alcohol for about a week at 37° C. The alcohol was separated from the worm material by filtration. The filtrate was evaporated and the residue was shaken with a small quantity of physiological salt solution, in which it dissolved, producing an opalescent solution. Tested on sheep red blood corpuscles this solution produced hemolysis. A quantity of the solution which hemolyzed 5 drops of a 5 per cent suspension of washed sheep corpuscles in about 2 hours at 37° failed to produce hemolysis on an equal quantity of blood corpuscles in 20 hours in a refrigerator (8°), thus showing that low temperatures paralyze the action of the hemolysin. Likewise, normal horse serum (2 drops) inhibited hemolysis of 5 drops of washed sheep corpuscles to which sufficient hemolytic solution had been added to cause hemolysis in the absence of

normal serum. The worm material from which the alcohol-soluble substance had been removed was dried and pulverized. A portion of this powder was added to washed sheep corpuscles but failed to produce any hemolytic effect, showing that the hemolytic substances of *Bustomum phlebotomum* are completely soluble in alcohol.

In a few experiments the effect of normal serum was tested with a view of determining when it contained bodies capable of inhibiting the action of *Bustomum phlebotomum* hemolysin. Washed rabbit corpuscles, belonging to a lot that were rapidly hemolyzed by a small quantity of the powder, resisted hemolysis in the presence of a few drops of normal rabbit serum.

The effect of heat on the hemolysin was found to be the same as the effect of heat on *Ancylostoma caninum* hemolysin. A salt-solution extract of fresh worms was completely inactivated by heating it for 40 minutes at 60° C.

## IX. EXPERIMENTS ON THE POSSIBLE PRESENCE OF ANTICOAGULINS IN HOOKWORMS

A series of experiments was performed with a view of determining whether the two species of hookworms discussed in the foregoing pages (*Ancylostoma caninum* and *Bustomum phlebotomum*) secrete a substance that has the power of inhibiting the coagulation of rabbit blood. Salt-solution extracts of fresh and dried material from the two species were tested as follows.

Into a series of tubes containing varying doses of extract, rabbit blood drawn directly from the marginal ear vein was allowed to drop. Each experiment was controlled by allowing an equal quantity of blood to drop into tubes containing physiological salt solution. So far as the rapidity of coagulation of the blood was concerned, appreciable but not very marked differences were detected between the test and control tubes. These experiments were performed on the blood of several rabbits with uniformly negative results.

Inasmuch as Loeb and his collaborators tested the anticoagulin from *Ancylostoma caninum* on dog blood and obtained positive results, it would appear that the writer's negative results may indicate that the anticoagulins in hookworms are either strictly specific for the blood of their host or that they are perhaps only relatively specific. Further data bearing on hookworm anticoagulin as well as anticoagulins from other nematodes are given in a separate paper (Schwartz, *1921*).

## X. EXPERIMENTS WITH EXTRACTS OF HAEMONCHUS CONTORTUS

Haemonchosis or stomach-worm disease is a disease of cattle and sheep due to the presence in the fourth stomach of a nematode parasite known as *Haemonchus contortus*. Young animals are especially susceptible to

stomach-worm disease, and among other symptoms they show those of a rather severe anemia. As in hookworm disease, the direct abstraction of blood by the parasites undoubtedly plays a part in bringing about the train of morbid symptoms associated with loss of blood, but that other factors are involved—namely, a chronic intoxication of the host by toxic substances liberated by the parasites—appears probable. Furthermore, it is by no means unlikely that as the suceptible animals grow older they become more or less immune to the effects of the parasites, although they are by no means immune to infestation with the worms. Whether the immunity is developed as a result of a previous infestation or whether it is a natural immunity associated with maturity is not known. In fact the clinical phase of haemonchosis is still an almost unexplored field in parasitology.

The following experiments were performed by the present writer with reference to the presence of a soluble hemotoxin in this parasite.

A number of specimens of *Haemonchus contortus* (about 100) that had been removed from a calf shortly after death were washed a number of times in physiological salt solution and kept in a refrigerator overnight. The following day the specimens were still alive. They were ground up in a mortar with a small quantity of physiological salt solution. The crushed material was transferred to a test tube and allowed to remain at room temperature for about two hours. The supernatant fluid was then tested on a 5 per cent suspension of washed sheep corpuscles. After 2 hours at 37° C. a number of tubes containing graded quantities of extracts and 5 drops of washed red blood cells showed no trace of hemolysis. The tubes were then transferred to a refrigerator, where they remained 18 hours longer. A faint trace of hemolysis was present in the tube containing the largest quantity of extract. The control tube was intact.

A second experiment of a similar nature was performed with another lot of fresh worms. In this case the extract was tested on washed sheep blood corpuscles, with negative results. Alcoholic specimens of *Haemonchus contortus* from sheep were washed in salt solution to remove traces of the alcohol and then dried at 37° C. The dried material was pulverized, and part of it was extracted in salt solution and tested on washed sheep corpuscles, with negative results. The remaining portion of the dried material was extracted in 95 per cent alcohol and the extract suspended in salt solution. Tested on sheep corpuscles, this extract likewise yielded negative results.

A number of fresh specimens of *Haemonchus contortus* were dried at 37° C. and pulverized in a mortar. Graded quantities of the powder were added to washed sheep blood corpuscles. After 2 hours at 37° followed by 18 hours in a refrigerator slight hemolysis was produced.

To one tube a small quantity of carbolized[1] horse blood serum was added. The serum inhibited hemolysis.

*Haemonchus contortus* powder was also tested on four samples of washed cattle blood cells. The results were slightly positive after 2 hours at 37° C. followed by 18 hours in a refrigerator.

Inasmuch as in the experiment described above washed red blood cells were used, a series of tests were performed in which unwashed defibrinated blood was used. In this series six samples of cattle blood were involved. The addition of various quantities of *Haemonchus contortus* powder yielded negative results after 3 hours at 37° C.

Summarizing, salt-solution extracts of *Haemonchus contortus* are very slightly hemolytic to sheep and cattle erythrocytes. The faint hemolytic property is preserved by drying. The weakly positive results obtained by experiments in vitro do not favor very strongly the view which has been commonly accepted as regards the secretion of a hemotoxin by *H. contortus*. It is quite possible, however, that the apparently weak hemolysin requires some activator which is supplied by the host blood. The fact that experiments in vitro were only slightly positive by no means precludes the possibility that an absorption by the host of the secretions of *H. contortus* is followed by a marked hemolysis. Another possibility, which has already been mentioned, is that only the blood of young animals is susceptible to the secretions of *H. contortus*. The subject requires further investigation.

## XI. EXPERIMENTS WITH TRICHURIS DEPRESSIUSCULA EXTRACT

A small series of experiments with an extract of *Trichuris depressiuscula* was performed as follows: About 60 specimens collected from several dogs were thoroughly washed in physiological salt solution and dried in an incubator. The dried specimens were then triturated and extracted in 3 cc. of salt solution overnight at 8° C. The clear filtrate was tested on rabbit and sheep erythrocytes. Five drops of a 5 per cent suspension of rabbit blood cells were hemolyzed by 3 drops of extract in about 2 hours at 37°. Equal mixtures of sheep erythrocytes and extract showed no hemolysis. The extract was boiled for about a minute, and after it had cooled it was tested on rabbit erythrocytes. It produced a faint indication of hemolysis, showing that boiling practically destroyed the hemolysin.

## XII. EXPERIMENTS WITH CESTODE HEMOLYSINS

It has already been stated that while an active hemolytic agent has been shown to occur in *Diphyllobothrium latum*, evidence that other species of tapeworms secrete hemolytic substances is rather incomplete. The presence of a hemolytic agent in *D. latum* is significant in view of the fact that this parasite is capable of producing a severe anemia under

[1] 0.25 per cent solution of carbolic acid in serum.

certain conditions that are not yet understood. Inasmuch as cestodes are not capable of causing anemia by direct abstraction of blood or by lacerating the mucosa, the etiological rôle of a hemotoxin, if such a substance can be demonstrated in forms that cause anemia, can hardly be denied. The discovery of Schaumann and Tallqvist (*1898*) and the subsequent studies of Tallqvist (*1907*) and Faust and Tallqvist (*1907*) with reference to the *D. latum* hemolysin are of great significance and mark the beginning of the study of the pathogenicity of parasitic worms from the point of view of intoxication. Despite the fact that *D. latum* appears to stand alone among cestodes capable of setting up a severe type of anemia, there is some evidence that other cestodes are also capable of bringing about anemia, perhaps not so intense as that produced by *D. latum*. Railliet (*1895*), Neveu-Lemaire (*1912*), Hutyra and Marek (*1913*), and other writers on veterinary parasitology state that cattle and sheep that are parasitized by tapeworms show clinical symptoms of anemia. Adult cestodes parasitic in these ruminants belong to the genera Moniezia and Thysanosoma. Only one species of the latter genus is known in the United States, namely, *Thysanosoma actinioides*, whereas several species of Moniezia occur in this country. Investigations by the present writer with reference to hemolysins in worms belonging to the genera Moniezia and Thysanosoma have yielded the following results.

A salt-solution extract of *Thysanosoma actinioides* powder made by adding the powder to salt solution and allowing the extract to remain at 8° C. for about 24 hours was found to be hemolytic to washed sheep blood cells and rabbit blood cells. In one experiment 150 mgm. of powder were extracted in 5 cc. of physiological salt solution overnight at a temperature of 8°. The supernatant fluid was filtered and the filtrate tested on washed rabbit blood cells. Equal parts of extract of suspension of corpuscles showed complete hemolysis after 2 hours at 37°. Further experiments with salt-solution extracts of dried material on washed sheep and rabbit blood corpuscles confirmed the presence of a soluble hemolysin in this parasite. Thus, an extract prepared by adding 0.2 gm. of powder to 2 cc. of salt solution was tested on rabbit and sheep blood corpuscles and yielded positive results. The action of the hemolysin was comparatively slow. To tubes each containing 5 drops of washed blood cells 5 and 10 drops, respectively, of the extract were added and incubated at 37° for 2 hours; hemolysis was not evident in the tubes. After an additional period of 18 hours during which the tubes were kept in a refrigerator hemolysis was complete in the tube to which 10 drops of extract had been added and marked but incomplete in the tubes to which only 5 drops of extract had been added. It should be stated in this connection that in several instances salt-solution extracts of dried *T. actinioides* were not destructive to red blood cells of sheep. Whether the red blood cells of certain animals are more resistant than

others, or whether the different extracts used in these experiments varied in their hemolytic content, has not been determined. At any rate, salt-solution extracts of *T. actinioides*, so far as the experiments referred to above are concerned, are not strongly hemolytic.

A quantity of *Thysanosoma actinioides* powder was extracted in four volumes of ether. The ether extract after it had been freed from all traces of ether was emulsified in physiological salt solution and tested on sheep blood corpuscles with positive results. After a second extraction of the powder in ether a quantity of powder free from the ether-soluble fraction was extracted in physiological salt solution, and this extract was found to be nonhemolytic. The remaining powder was extracted in 95 per cent alcohol. After filtration the alcohol was evaporated, and the residue, which had a waxy appearance and consistency, was dissolved in physiological salt solution and tested on sheep red blood cells with positive results. Boiling did not destroy the hemolytic potency of this extract; neither did cold inhibit its activity. Normal horse serum inhibited its action completely.

After alcohol extraction the powder was extracted in physiological salt solution and tested on sheep red blood cells. It was only faintly hemolytic.

Another lot of powdered *Thysanosoma actinioides* was extracted in 95 per cent alcohol three times in succession, each extraction lasting 48 hours. After the last extraction only a slight residue was left when the alcohol had completely evaporated. The residues were dissolved in physiological salt solution and tested on sheep and rabbit blood cells with positive results. Boiling did not destroy them and low temperatures had no inhibiting effect on them. The powder freed from the alcohol-soluble fraction was extracted in salt solution, and this extract was nonhemolytic.

It may be concluded, therefore, that a hemolysin is present in *Thysanosoma actinioides*, soluble to some extent in physiological salt solution and completely soluble in alcohol. Ether extracts of *T. actinioides* are hemolytic, but worm material freed from ether-soluble fractions still retain the hemolytic agent. That substances other than fatty acids are involved in the hemolytic effects of *T. actinioides* extracts is evident, since the ether extracts remove whatever fatty acids are present in the worms. The alcohol-soluble and ether-insoluble fraction of *T. actinioides* resembles rather closely tissue lysins so far as the chemical and physiological properties of tissue lysins are known. Noguchi (*1907*) found that tissue lysins are soluble in 95 per cent alcohol, are not removed by ether extraction, and that they have the chemical properties of soluble soaps. In common with the latter they are destructive to red blood cells even at 0° C., are neutralized by normal serum, and are resistant to boiling. While the chemical nature of the ether-insoluble and alcohol-soluble fraction of *T. actinioides* has not been determined, its resem-

blance to tissue lysins appears to be very close. The *Ascaris lumbricoides* hemolysin as well as the *Bustomum phlebotomum* and *Ancylostoma caninum* hemolysins are not active at low temperatures, as shown elsewhere in this paper.

Experiments with a species of Moniezia similar to those performed with *Thysanosoma actinioides* have yielded negative results. The addition of various quantities of powdered Moniezia material to suspension of washed red blood cells of rabbit and sheep produced no destructive action on the cells. A salt-solution extract of Moniezia powder was likewise nonhemolytic when tested on washed sheep blood cells. An ether extract was only slightly hemolytic, but after removing from the ether extract the acetone-insoluble fraction, presumably lecithin, its hemolytic potency was no longer manifest. The acetone-insoluble fraction had no destructive effect on sheep blood corpuscles. A quantity of Moniezia powder freed from the ether-soluble fraction by repeated extraction with ether was extracted for 72 hours in 95 per cent alcohol at 38° C. The alcohol was separated from the alcohol-insoluble powder by filtration and evaporated. The residue was taken up in physiological salt solution, in which it was only partly soluble, the insoluble portion forming a coarse suspension. This solution had a decidedly acid reaction. Tested on washed sheep red blood corpuscles, it produced no hemolytic effect.

## XIII. RESULTS OF EXPERIMENTS

The data presented in the foregoing pages have already been summarized in connection with each separate topic. The discussion which follows is for the purpose of correlating, comparing, and differentiating the results obtained with various species of parasitic worms that have been referred to in this paper, and to consider the general bearings that the results have on the nature of parasitic infection.

Hemotoxins present in parasitic worms contain one or more active principles. Of the latter, hemolysins stand out as of prime importance. Hemagglutinins and anticoagulins may be associated with hemolysins.

Hemagglutinins have thus far been observed in *Diphyllobothrium latum* by Tallqvist (*1907*) and in *Ascaris lumbricoides* by the present writer. Tallqvist describes the hemagglutinin from *D. latum* as a water-soluble, alcohol- and ether-insoluble substance, decidedly thermostabile. The hemagglutinin observed by the present writer in extracts of *Ascaris lumbricoides* is resistant to heat and soluble in lipoid solvents, such as ether and alcohol, as well as in physiological salt solution. It is, therefore, quite a different substance from the agglutinin of *D. latum*. Anticoagulins have been found in species of Strongylus (Weinberg, *1908*), in the larvae of Gastrophilus (Weinberg, *1908*), in species of Ascaris (Weil and Boyé, Leroy, and the present writer), in *Ancylostoma caninum* (Loeb

and his collaborators), and in several other species by the present writer (*1921*.) The anticoagulin in *Ancylostoma caninum* is the most active of the anticoagulins observed in parasitic worms and is doubtless a factor in the anemia that is present in hookworm disease. The anticoagulin of *Ascaris lumbricoides* has but a feeble action, so far as available experimental data show.

Hemolysins from parasitic worms, so far as they have been described in the literature, have certain properties in common with hemolysins of bacterial origin as well as with hemolysins that have been obtained from normal tissues by Korschum and Morgenroth, Noguchi, and others. These properties may be characterized as nonspecificity in action and relative simplicity of structure as compared with hemolysins that may be artificially produced in animals by immunization with red blood corpuscles. The experiments recorded in this paper do not in any case contradict these facts. Different species of blood corpuscles may show differences in resistance to hemolytic extracts of parasitic worms, but absolute resistance of any species of corpuscles has not been established. Similarly, extracts from different parasitic worms differ in their resistance to heat, but once their potency has been destroyed it can not be reactivated by normal serum. The only apparent contradiction to this statement is the result of a small series of experiments of Garin with *Graphidium strigosum*, which, as has already been indicated, can not be accepted as conclusive in view of the small number of experiments. Hemolysins produced in an animal as a result of immunization with red blood corpuscles, are, as is well known, specific in their action, affecting only corpuscles against which the animal has been immunized, and complex in structure, since they act in combination with complement and may be reactivated by normal serum after the complement has been destroyed.

So far as their resistance to heat is concerned, hemolysins from parasitic worms differ considerably. Heat-resisting hemolysins have been recorded by Tallqvist from *Diphyllobothrium latum*, by Weinberg from species of Strongylus, and by the present writer from *Ascaris lumbricoides* and *Thysanosoma actinioides*. Hookworm hemolysins from worms of the genera Ancylostoma, Necator, Bustomum, and the hemolysin from *Trichuris depressiuscula* are not as resistant to heat. The relatively thermolabile hemolysins from these parasites resemble in this respect bacterial hemolysins, whereas the thermostabile hemolysins resemble in this respect tissue extracts.

The solubility of hemolysins from parasitic worms in lipoid solvents, especially in alcohol, is another property that they have in common with tissue lysins. A property of the latter is also the nonimpairment of their activity at low temperatures, even at 0° C. So far as the results of experiments recorded in this paper are concerned, hemolysins from

worms belonging to the genera Ascaris, Ancylostoma, and Bustomum are inhibited at 8°. The hemolytic effect of *Thysanosoma actinioides* extract is not inhibited at this temperature, however. This fact is important and clearly differentiates hemolysins of nematodes from the hemolysin of *T. actinioides*. In this respect, too, nematode hemolysins resemble bacterial hemolysins.

Finally, the action of hemolysins from parasitic worms is inhibited by normal serum. The antilytic property of the serum is thermostabile (Weinberg, *1908*, and the experimental results obtained by the present writer). Tissue lysins, too, are inhibited by normal serum. Certain bacterial hemolysins are similarly susceptible to normal serum.

On the basis of this discussion nematode hemolysins may be characterized as relatively simple substances, thermolabile or thermostabile, depending on the species from which they are obtained, inactive at low temperatures (6° to 8° C.), inactive in the presence of normal serum, nonspecific, soluble in alcohol and in physiological salt solution.

Cestode hemolysins, so far as they have been investigated, are relatively simple bodies, thermostabile, active at low temperatures, inactive in the presence of normal serum, nonspecific, soluble in alcohol.

The question naturally arises whether toxic products from parasitic worms are liberated from the bodies of the latter and get into the circulation of the host. Blanchard (*1905*), while accepting the evidence in favor of the view that parasitic worms elaborate toxic products, appears to doubt the etiological significance of these toxic substances because of the possibility that they are either not liberated by the worms or if liberated may be thrown out of the body before they can injuriously affect the host. The available evidence on this question appears to indicate that hosts harboring parasitic worms actually absorb the toxic products of the latter. The serological evidence in favor of this view has already been referred to. It may be added that the fact reported by Guerrini (*1908*) with reference to the presence of hemolysins in the blood serum of hosts harboring *Fasciola hepatica* and the findings of De Blasi that hemolysins occur in the blood serum of hosts harboring *Ancylostoma duodenale* tend to confirm the belief that parasites liberate their toxic secretions and that these secretions get into the circulation of the host. Weinberg (*1908*) has made some interesting observations on the tissues of hosts harboring parasitic worms which argue directly in favor of the absorption by the host of toxic products liberated by the worms. Weinberg examined histologically the organs of 32 horses infested with strongyles and obtained the following results: In the blood vessels he found a large number of mononuclear leucocytes containing iron granules. He also found similar granules in the spleen, liver, in the conjunctival tissue, in the Malpighian tubules and in the convoluted tubules of the kidneys, and in the canals of the right kidney. Histological examinations by the same investigator of organs from 30 monkeys infested with a species of

Œsophagostomum yielded similar results. In another paper Weinberg (*1909*) records that the injection of extracts of worms of the genus Strongylus into guinea pigs leads to a pigmentation of the spleen but seldom of the liver. From this, it appears that erythrocyte destruction takes place in animals that harbor hemotoxin-producing parasitic worms and that the disintegration products of the erythrocytes are ingested by leucocytes, arrested in certain organs, and eliminated through the excretory system.

Whether the hemotoxic substances from parasitic worms are liberated during the normal life of the worms, or whether they are liberated only when worms sicken or degenerate, as appears to be the case in *Diphyllobothrium latum*, can not as yet be stated with certainty. In the case of *D. latum* the view that only certain specimens secrete the hemolysin has been advanced by a number of investigators, especially by Leichtenstern (*1896*). Tallqvist's experiments show that hemolysins are present in specimens of *D. latum* expelled from patients that show no symptoms of anemia as well as in specimens obtained from cases of severe anemia. Tallqvist's hypothesis that the hemolysin is eliminated when the worms disintegrate finds confirmation in numerous cases in which eggs of *D. latum* are present in the feces of patients, and anthelmintic medication fails to expel any worms and merely yields a mass of eggs. Another factor which may be of importance, and which, so far as the present writer is aware, has been entirely overlooked, is the fact that certain individuals may lack antilytic constituents in their blood and are thus susceptible to the toxin which other individuals are capable of neutralizing. That the antilytic properties of the blood may under certain conditions be absent is probable from the observations of Noc (*1908*) with reference to hookworm disease. Whether the observations with reference to *D. latum* are applicable to other parasitic worms, especially to nematodes, can not in the light of our present knowledge be stated with any degree of certainty. That parasites may die in the intestine or other location and disintegrate before they are eliminated from the body of the host is by no means improbable. Cultures of larvae of parasitic worms in vitro show that bacteria may kill the worms, and that the latter undergo degenerative changes, such as complete internal disorganization, quite rapidly. That worms may be attacked by bacteria and other organisms in the body of the host is by no means improbable. Weinberg has in fact described what appears to be a disease in worms belonging to the genus Ascaris, which is characterized by the presence of certain pigmented spots that are clearly visible through the cuticle. The present writer has observed this condition in specimens of *Ascaris lumbricoides* on several occasions.

Whether parasitic worms liberate their toxic secretions during life or whether these substances partake of the nature of endotoxins and are not liberated from the bodies of the worms unless the latter disintegrate

is still open to speculation, but the view that toxic substances from parasitic worms are of etiological significance in parasitic diseases is supported by convincing evidence.

## XIV. SUMMARY

Extracts of *Ascaris lumbricoides* contain active substances that affect blood deleteriously. The hemolysin which these extracts contain is a thermostabile, nonspecific, alcohol-soluble substance which appears to be rather firmly bound to the cells of the parasite, presumably to the cells of the intestine in which it is elaborated. The hemolytic potency of extracts of *A. lumbricoides* is not due solely to fatty acids, since chemical fractions of the worms from which the fatty acids have been removed by ether extraction are hemolytic. The hemolysin is neutralized by normal blood serum.

The body fluid of *A. lumbricoides* shortly after removal from the host contains oxyhemoglobin and is nonhemolytic. It acquires hemolytic powers, however, as the worms are kept alive in vitro for a few days, and loses at the same time its oxyhemoglobin content.

Body fluid from fresh specimens of *Ascaris lumbricoides* does not activate a hemolytic system, and alcohol-soluble fractions of the worms from which ether-soluble substances have been removed does not act as complement in combination with inactivated normal guinea-pig serum.

The hemagglutinin from *Ascaris lumbricoides* is a salt-solution-soluble substance and has special affinities for rabbit blood cells. Unlike the hemolysin, its action is not hindered by low temperatures (6° to 8° C.).

*Ascaris lumbricoides* secretes a substance that inhibits the coagulation of blood. This substance is present in the body fluid of the worm and has but a comparatively slight potency.

*Ancylostoma caninum* secretes a nonspecific hemolysin, soluble in salt solution, relatively thermolabile and inactive at low temperatures. Normal blood serum inhibits the action of the hookworm hemolysin.

*Bustomum phlebotomum* secretes a hemolysin having properties similar to that of *Ancylostoma caninum*. This hemolysin is completely soluble in alcohol.

Salt-solution extracts of *Haemonchus contortus* have but a feeble hemolytic action.

Salt-solution extracts of *Ancylostoma caninum* and of *Bustomum phlebotomum* do not inhibit the coagulation of rabbit blood to any marked degree.

A weak hemolytic substance is present in extracts of *Trichuris depressiuscula*.

*Thysanosoma actinioides* contains an alcohol-soluble hemolysin. Alcohol-soluble fractions of *T. actinioides* from which the ether-soluble fraction has been removed are hemolytic, showing that substances other than fatty acids are involved. The hemolysin from this cestode is active at 8° C.

and is neutralized by normal blood serum. Extracts of a species of Moniezia similar to those of *Thysanosoma actinioides* are nonhemolytic.

The view that hemolysins and other hemotoxic secretions of parasitic worms are of etiological importance in parasitic diseases appears to be well founded.

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# ASH CONTENT OF THE AWN, RACHIS PALEA, AND KERNEL OF BARLEY DURING GROWTH AND MATURATION

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## INTRODUCTION

The ash determinations here assembled were made with two primary objects in view. Earlier studies had shown that in the varieties under observation the awns possessed a physiological function. When they were removed the kernel development was retarded and the spike became brittle through the greater ash deposit in the rachis. In order to see if usable variations existed in the amount of ash deposited in the rachises and awns, a considerable number of varieties were studied.

The previous experiments were not as complete as was desired. Mechanical difficulties had prevented the taking of samples to the point of absolute maturity. There thus existed a possible doubt as to the nature of the changes in the days immediately following the date when kernel sampling became impossible. The determination of ash in the awns and rachises was, therefore, continued for some time after maturity in one series of varieties at Chico, Calif.

The results point a possible way to the securing of desirable non-shattering awnless and hooded varieties. They also throw some light on the ash content of the kernel during growth.

## MATERIAL USED

Material for the study of ash in the barley spike was collected from several sources. Two series of samples originated at Aberdeen, Idaho. The awns, rachises, and paleas were obtained from the irrigation plots, the kernel studies of which were previously reported. In this series and the one from Minnesota the glumes were forcibly removed from the kernels. To eliminate the possible effect of imperfect separation when the glumes were thus removed, the kernels from a naked barley grown at Aberdeen were included for comparison.

Two lots of samples were grown at Chico, Calif. The first of these consisted of a collection of varieties embracing a wide range of botanical characters. The second consisted of a lesser number of varieties, which were allowed to stand in the field for a time after ripening. Frequent samples were taken, and the change of ash after maturity was determined.

Further use also was made of the data from an experiment carried on at St. Paul, Minn. Ash determinations were made on a number of varieties grown at Arlington, Va., the detailed results of which are not included.

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ASH OF THE AWNS

The awns of barley contain a very high proportion of ash. One of the most finely divided carbons known has been secured from barley awns. This extremely fine division is probably caused by the high percentage of ash. The ash is deposited during the time the kernel is developing. At the time of their emergence the awns contain little ash and are very flexible.

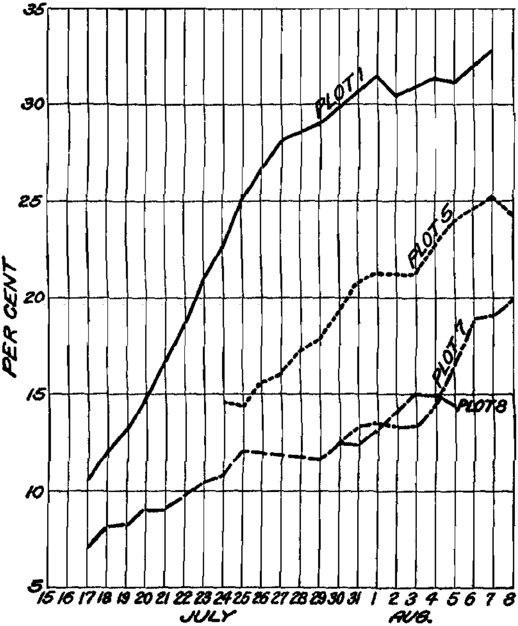


FIG. 1.—Percentage of ash, by progressive three-day averages, in the awns of Hannchen barley grown on plots variously irrigated at Aberdeen, Idaho, in 1917.

The ash at flowering time and for a few days immediately following usually runs from 4 to 8 per cent of the dry matter. As may be seen in Table I, the awns of the Hannchen variety may sometimes contain a slightly higher percentage at flowering time. Varieties of this type, however, contain more ash than do those of most other types of barley. The increase in the ash content after flowering is very regular. The daily

increment is quite uniform. In figure 1 it will be seen that within the variety the rate of deposit has a direct relationship with the amount of moisture in the soil and probably with the amount of water transpired. On plot 8, which was not irrigated after flowering, the plants were suffering from lack of water during most of the time the kernels were developing. This lack of water is reflected in the percentage of ash in the awns. The awns on this plot never contained as high as 16 per cent of ash. The uniformity of the deposit was in no wise affected. The daily increment, however, was less than on plot 1, which received ample irrigation. The normal ash content of the awn of Hannchen barley when grown in the western United States is over 30 per cent at maturity. As will be seen, the awns on the spikes from plot 1 reached this percentage several days before maturity. Plots 2 to 7 received the same treatment as plot 8 until the time of their final irrigation. Only one irrigation after flowering was given to any plot except plot 1. The irrigation occurred on the day the first ash determination was reported. Following the application of water there was an acceleration in the rate of deposit of ash in plots 2 to 6, inclusive. The barley on plot 7 was maturing when the water was applied. The leaves had begun to wither and the awns were almost color-free at the time of the irrigation. The rate of deposit was not materially increased over the rate on plot 8. The maturation was, however, delayed, and the final ash content of the awns was decidedly greater than in plot 8.

TABLE I.—*Percentage of ash in the awns of Hannchen barley grown on eight different plots variously irrigated, at Aberdeen, Idaho, in 1917*

| Date. | Plot. 1 | Plot 2. | Plot 3. | Plot 4. | Plot 5. | Plot 6. | Plot 7. | Plot 8. |
|---|---|---|---|---|---|---|---|---|
| July 16 | 9.4 | 16.2 | ...... | ...... | ...... | ...... | ...... | 6.0 |
| 17 | 10.8 | 12.5 | ...... | ...... | ...... | ...... | ...... | 7.8 |
| 18 | 11.6 | 14.2 | 8.7 | ...... | ...... | ...... | ...... | 7.3 |
| 19 | 13.9 | 16.1 | 14.0 | ...... | ...... | ...... | ...... | 9.3 |
| 20 | 14.0 | 14.7 | 15.7 | 7.8 | ...... | ...... | ...... | 8.2 |
| 21 | 16.5 | 16.5 | 18.4 | 7.9 | ...... | ...... | ...... | 9.5 |
| 22 | 19.6 | 17.7 | 18.8 | 9.7 | ...... | ...... | ...... | ...... |
| 23 | 20.0 | 19.3 | 19.8 | 15.1 | 13.8 | ...... | ...... | 9.4 |
| 24 | 23.8 | 20.2 | 21.9 | 16.3 | 13.4 | ...... | ...... | 12.2 |
| 25 | 23.9 | 19.8 | 18.1 | 15.0 | 16.5 | ...... | ...... | 10.7 |
| 26 | 27.5 | 20.6 | 19.9 | 18.7 | 13.4 | 10.3 | ...... | 13.2 |
| 27 | 28.6 | 21.6 | 21.9 | ...... | 17.2 | 13.2 | ...... | 11.8 |
| 28 | 25.4 | 20.4 | 21.9 | ...... | 17.7 | 16.0 | ...... | 10.5 |
| 29 | 28.4 | 20.4 | ...... | ...... | 17.0 | 12.2 | 11.1 | 13.0 |
| 30 | 30.0 | 21.5 | ...... | ...... | 18.9 | 15.4 | 12.6 | 11.7 |
| 31 | 31.2 | 21.1 | ...... | 20.5 | 22.1 | 18.4 | 13.9 | 12.4 |
| Aug. 1 | 30.7 | 22.4 | 26.5 | 23.1 | 21.7 | 18.3 | 13.5 | 13.1 |
| 2 | 32.7 | 21.2 | 24.2 | 23.9 | 19.9 | 21.5 | 13.1 | 13.9 |
| 3 | ...... | ...... | 26.2 | 22.6 | 21.5 | 23.2 | 13.2 | 15.4 |
| 4 | 28.2 | ...... | 26.3 | 20.2 | 22.1 | 18.0 | 13.8 | 15.6 |
| 5 | 33.1 | 29.1 | 32.9 | 20.4 | 24.5 | 21.7 | 16.6 | 13.7 |
| 6 | ...... | ...... | 30.1 | 19.7 | 25.4 | 22.5 | 19.0 | 13.8 |
| 7 | 29.1 | ...... | 27.8 | 20.0 | 24.0 | 26.2 | 21.2 | ...... |
| 8 | 33.1 | 28.2 | 23.2 | ...... | 26.2 | 22.7 | 17.2 | ...... |
| 9 | ...... | ...... | 32.3 | 25.7 | 22.7 | 32.1 | 21.5 | ...... |

TABLE II.—*Percentage of ash in awns, rachises, and kernels of eight varieties of barley grown at Chico, Calif., and sampled on 10 different dates*

PERCENTAGE OF ASH IN AWNS

| C. I. No. | Variety. | June 6. | June 9. | June 13. | June 16. | June 20. | June 25. | June 27. | June 30. | July 7. | July 14. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 531 | Hannchen | 36.7 | 36.1 | 34.4 | 31.8 | 35.5 | 34.6 | 31.5 | 33.2 | 32.8 | 35.8 |
| 234 | Nepal[1] | 16.6 | ...... | ...... | ...... | ...... | ...... | 16.4 | 13.8 | 15.5 | 16.3 |
| 257 | Tennessee Winter | 29.1 | ...... | 32.1 | 33.2 | 34.4 | 34.1 | 34.1 | 35.3 | 34.3 | 30.7 |
| 916 | Odessa | 34.3 | 35.8 | 35.6 | 34.6 | 33.1 | 35.3 | 36.1 | 37.4 | 37.2 | 35.5 |
| 261 | Mariout | 34.1 | 34.3 | 34.5 | 36.7 | 35.2 | 37.2 | 33.5 | 32.6 | 34.0 | 33.7 |
| 195 | Smyrna | 32.0 | 30.1 | 35.5 | 29.5 | 35.0 | 29.0 | 32.7 | 29.5 | 31.2 | 35.2 |
| 690 | Coast | 31.9 | 31.8 | 26.6 | 32.8 | 33.0 | 30.8 | 32.5 | 32.2 | 31.7 | 32.4 |
| 652 | Poda | 27.3 | 30.0 | 28.4 | 31.0 | 33.6 | 32.1 | 32.4 | 33.4 | 32.5 | 33.5 |

PERCENTAGE OF ASH IN RACHISES

| C. I. No. | Variety. | June 6. | June 9. | June 13. | June 16. | June 20. | June 25. | June 27. | June 30. | July 7. | July 14. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 531 | Hannchen | 12.1 | 11.1 | 11.7 | 10.7 | 10.2 | ...... | 9.5 | 10.0 | 11.4 | 11.5 |
| 234 | Nepal | 9.5 | ...... | ...... | ...... | ...... | ...... | 9.5 | 9.9 | 10.5 | 9.8 |
| 257 | Tennessee Winter | 7.4 | 10.0 | 9.6 | 8.1 | 7.9 | 9.8 | 9.8 | 8.9 | 8.3 | 8.8 |
| 916 | Odessa | 8.1 | ...... | 8.5 | 6.6 | 7.7 | 7.5 | ...... | 8.7 | 9.6 | 8.0 |
| 261 | Mariout | 7.7 | 7.8 | 7.6 | 8.9 | 8.6 | 9.1 | 8.5 | 7.8 | 8.6 | 8.4 |
| 195 | Smyrna | 7.7 | 7.7 | 8.2 | 7.2 | 8.4 | 6.8 | 7.1 | 7.7 | 6.8 | 7.8 |
| 690 | Coast | 6.0 | 5.4 | 5.0 | 6.3 | 6.8 | 6.2 | 6.8 | 6.4 | 6.9 | 6.0 |
| 652 | Poda | 5.1 | 5.4 | 5.4 | 5.5 | 5.1 | 5.7 | 5.8 | 6.0 | 6.0 | 6.1 |

PERCENTAGE OF ASH IN KERNELS

| C. I. No. | Variety. | June 6. | June 9. | June 13. | June 16. | June 20. | June 25. | June 27. | June 30. | July 7. | July 14. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 531 | Hannchen | 3.3 | 3.2 | 3.6 | 3.4 | 3.4 | 3.5 | 3.3 | 3.6 | 3.3 | 3.4 |
| 234 | Nepal[2] | 1.8 | ...... | ...... | ...... | ...... | ...... | 1.9 | 1.8 | 2.1 | 2.0 |
| 257 | Tennessee Winter | 3.2 | 3.4 | 3.5 | 3.1 | 3.2 | 3.2 | 3.3 | 3.2 | ...... | 3.0 |
| 916 | Odessa | 3.6 | 3.6 | 3.4 | 3.4 | 3.4 | 3.6 | 3.4 | 3.6 | 3.6 | 3.6 |
| 261 | Mariout | 3.0 | 3.1 | 2.9 | 3.2 | 3.1 | 3.2 | 3.1 | 3.1 | 3.1 | 3.1 |
| 195 | Smyrna | 2.7 | 2.7 | 3.1 | 2.8 | 3.1 | 3.8 | 2.8 | 3.3 | 2.8 | 3.1 |
| 690 | Coast | 3.0 | 3.2 | 3.0 | ...... | 3.1 | 2.9 | ...... | 3.1 | 3.5 | ...... |
| 652 | Poda | 2.8 | 2.9 | 2.8 | 2.9 | 3.0 | 2.9 | 3.1 | 3.0 | 2.8 | 2.9 |

[1] Hoods.

[2] Naked kernels.

Since the studies on irrigation were not carried beyond the stage of actual maturity, there was some question as to whether the awn had ceased to accumulate ash when the studies were terminated. It was impossible to carry these particular samples further, as this was a study of kernel growth and the paleas could not be stripped from the kernels after mechanical loss of water had commenced. In order to determine whether there was a later transfer of ash a series of varieties was allowed to stand in the field for six weeks after maturity, at Chico, Calif. Samples were taken, commencing at about the stage where they were discontinued at Aberdeen. These results are reported in Table II. It will be seen that there was very little change of ash content after the growth of the

kernels had been completed. The changes indicated in the table are probable variations of individual samples, inasmuch as the average of all the samples showed no consistant change.

TABLE III.—*Percentage of ash in the rachises, awns, and kernels of 39 varieties of barley grown at Chico, Calif., 1917*

| C. I. No. | Variety. | Description. | Percentage of ash. | | | |
|---|---|---|---|---|---|---|
| | | | Rachis. | Awn. | Grain. | Hoods. |
| 1079A | Chinerne | Black awnless 6-rowed | 13.6 | ..... | 3.5 | ..... |
| 1289 | Horsford | Hooded 6-rowed | 13.1 | ..... | 3.2 | 17.4 |
| 678 | Hanna | Lax 2-rowed | 13.0 | 35.3 | 3.2 | ..... |
| 1045 | Envoy | Dense 6-rowed | 11.4 | 20.9 | 3.7 | ..... |
| 1097 | Black Hull-less | Naked 6-rowed | 10.2 | 21.2 | 2.7 | ..... |
| 1449 | Hadaka | Short-awned naked 6-rowed | 10.1 | 28.9 | 2.3 | ..... |
| 1041 | Thomas | Naked 6-rowed | 10.1 | 23.5 | 2.4 | ..... |
| 1094 | Crocket | Shattering 6-rowed | 9.5 | 27.6 | 3.8 | ..... |
| 1284 | Feline | Smooth-awned 6-rowed | 9.4 | 27.9 | 3.0 | ..... |
| 679 | Franconian | Lax 2-rowed | 9.3 | 28.7 | 2.6 | ..... |
| 1046A | Temple | Dense naked 6-rowed | 9.2 | 22.5 | 2.2 | ..... |
| 1236 | Abyssinian | Dense deficient 2-rowed | 9.1 | 24.7 | 3.5 | ..... |
| 1061 | Consul | Lax 6-rowed | 8.7 | 24.2 | 3.5 | ..... |
| 914 | Italian | Lax 2-rowed | 8.4 | 23.9 | 3.3 | ..... |
| 1072 | Squiers | Lax purple 6-rowed | 8.4 | 18.9 | 3.4 | ..... |
| 1451 | Carrol | ....do | 8.1 | 20.4 | 3.4 | ..... |
| 187 | Svanhals | Dense 2-rowed | 8.1 | 20.1 | 2.3 | ..... |
| 927 | Odessa | Dense 6-rowed | 8.1 | 23.9 | 2.3 | ..... |
| 1060 | Coolie | ....do | 8.1 | 20.1 | 3.4 | ..... |
| 1450 | Mochi | Long-awned naked 6-rowed | 8.0 | 20.4 | 2.4 | ..... |
| 1281 | Welch | Smooth-awned 6-rowed | 7.8 | 31.5 | 3.5 | ..... |
| 1296 | Kitchin | ....do | 7.7 | 31.1 | 2.9 | ..... |
| 1059A | Filer | Dense 6-rowed | 7.6 | 17.2 | 3.4 | ..... |
| 1038 | Judith | Lax 6-rowed | 7.2 | 19.3 | 3.2 | ..... |
| 1121 | Hanchamont | Lax 2-rowed | 7.2 | 23.2 | 2.7 | ..... |
| 1121 | ....do | ....do | 7.2 | 20.4 | 2.5 | ..... |
| 669B | Abyssinian | Purple deficient | 7.1 | 23.1 | 2.9 | ..... |
| 957 | Oderbrucker | Lax 6-rowed | 7.0 | 26.3 | 2.4 | ..... |
| 973 | Red River | ....do | 6.9 | 19.5 | 2.4 | ..... |
| 972 | Luth | ....do | 6.8 | 20.8 | 2.5 | ..... |
| 1076B | Venezuela | ....do | 6.8 | 25.2 | 2.8 | ..... |
| 190 | Beldi | ....do | 6.5 | 33.9 | 3.0 | ..... |
| 1074B | Algeria | ....do | 6.1 | 26.7 | 2.8 | ..... |
| 996 | Rasput | ....do | 6.1 | 28.7 | 3.4 | ..... |
| 1058 | Gobi | ....do | 5.7 | 17.2 | 2.7 | ..... |
| 1098B | Kurof | Lax 2-rowed | 5.6 | 13.4 | 3.5 | ..... |
| 1297 | Claudia | Smooth-awned 6-rowed | 5.5 | 30.2 | 2.8 | ..... |
| 1283 | Catto | ....do | 5.2 | 34.0 | 3.1 | ..... |
| 1307 | Cheddar | ....do | 4.8 | 30.1 | 3.3 | ..... |

Single samples from a larger number of varieties were taken at Chico the same year. These samples were not taken until it was evident that all growth in the plant had ceased. The results are reported in Table III. The list of varieties included almost all the major botanical variations of barley. The table is arranged in order of the ash content of the rachis, the ash of the awns being given in the second column. It will be noticed that the greater number of varieties have an ash content very much lower than the Hannchen at Aberdeen. It is not thought that much of

this is due to environment, although part of it probably is. The water available for the plants at Chico was less than at Aberdeen, since the plots at Chico were not irrigated. Although the Hannchen variety was not included in this nursery series, C. I. 679, Franconian, is of the same general type as Hannchen, and Hanna 678 probably is even more closely related. In Table II samples of Hannchen from a neighboring plot are reported, and these do not differ materially from those grown at Aberdeen. The agreement between the results at Chico and Aberdeen is close when it is realized that varieties do vary a great deal according to their environment, as was evident in the results from the irrigation experiments. Varieties grown in the East, under humid conditions where the ash content of the soil is very low, have a much lower percentage of ash than do those from the West. The determinations from Arlington, Va., are not reported, but they show far less ash than those from either Chico or Aberdeen. Despite the variation in the ash content, the awns of different varieties seem to maintain the same relationship. The varieties which are high in ash under the arid conditions of the West are also the ones which are highest in ash at Arlington, even though the ash content at Arlington may be only half that of the western-grown samples.

Varieties which have a low ash content in the rachis do not necessarily have a low ash content in the awns. The awn itself does not have the same ash content throughout its length. Variation in individual samples can easily come about through the loss of the tips of the awns in the field. In Table IV are given the results of determinations made on the basal, middle, and apical portions of the awns of three barleys from Chico, Calif. The ash content of the tip is much greater than that of the base. In the Hannchen and Tennessee Winter varieties, the ash reaches 40 per cent of the dry weight in the tips of the awns. The bases of the awns in the Coast variety were low in ash as compared with those of the Hannchen and Tennessee Winter varieties. This may have some connection with the fact that the awns of the Coast variety do not break cleanly from the grain in thrashing.

TABLE IV.—*Percentage of ash in the tip, middle, and basal portions of awns in three varieties of barley grown at Chico, Calif., in 1917*

| C. I. No. | Variety. | Date taken. | Percentage of ash. | | |
|---|---|---|---|---|---|
| | | | Tip. | Middle. | Base. |
| 257 | Tennessee Winter | June 6 | 34.3 | 35.7 | 29.7 |
| | | July 14 | 37.8 | 36.5 | 31.3 |
| 690 | Coast | June 6 | 29.6 | 28.4 | 23.8 |
| | | July 14 | 34.5 | 32.2 | 26.4 |
| 531 | Hannchen | June 6 | 41.4 | 39.6 | 33.2 |
| | | July 14 | 40.3 | 37.9 | 32.5 |

## ASH IN THE RACHIS

The deposit of ash in the rachis of the barley spike is less easily interpreted than is the ash in the awns. The awns serve as a place of deposit, probably for ash excluded from the cell sap. The rachis, on the other hand, is a conductive organ through which passes the nourishment of the various kernels and the water which is transpired from the awns. The daily deposit of ash in the rachis is confusing. Although a large number of analyses were made they are not reported, as no plausible explanation could be offered for the fluctuations. The general trend of the results is indicated in figure 2.

In 1917, in plot 8, which received no irrigation after flowering, the ash gradually increased from about 2 per cent at flowering time to about 7 per cent at maturity. In this case there were no large fluctuations. Where irrigation water was applied, the ash content was considerably increased. Although this increase was exhibited on all plots, in many cases the increases were irregular, fluctuating and not easily explained. The results in 1916 were more uniform and showed a gradual increase from flowering to maturity, the content reaching 11 to 14 per cent at that time. In 1917, on the irrigated plots, the content at maturity ranged from 12 to 18 per cent.

While the drop in ash in plot 1 is doubtless exaggerated by the accident of sampling, most of the large fluctuations in the daily samples of 1917 are not thought to be errors of determination. On the plots where the water content was low the fluctuations either did not occur or were small. The analyses of the awns and rachises were made from the same samples at the same time and in the same way. Those of the awns were satisfactory. It is probable that the variations in the ash of the rachises were due to some relationship of soil water and the rate of transpiration.

In a previous paper[1] it was shown that the removal of the awns resulted in an increase of the ash content in the rachis of an awned barley. From this it was inferred that the rachises of awnless barleys were likely to be high in ash. It was known that awnless and hooded varieties shattered badly in the field. It was to discover varietal differences, if such existed, that the samples were taken which are reported in Table III. As previously stated, the experiment included not only varieties which differed in the character of the awns but in many other taxonomic characters as well. It was found that the ash content varied greatly with the variety. As these samples were grown in California, the percentage of ash is higher than if the samples had been grown in the more humid districts. In all determinations made on barleys grown in Minnesota and at Arlington, Va., under humid conditions and where

[1] HARLAN, Harry V., and ANTHONY, Stephen. DEVELOPMENT OF BARLEY KERNELS IN NORMAL AND CLIPPED SPIKES AND THE LIMITATIONS OF AWNLESS AND HOODED VARIETIES. *In* Jour. Agr. Research, v. 19, no. 9, p. 431-472, 15 fig. 1920.

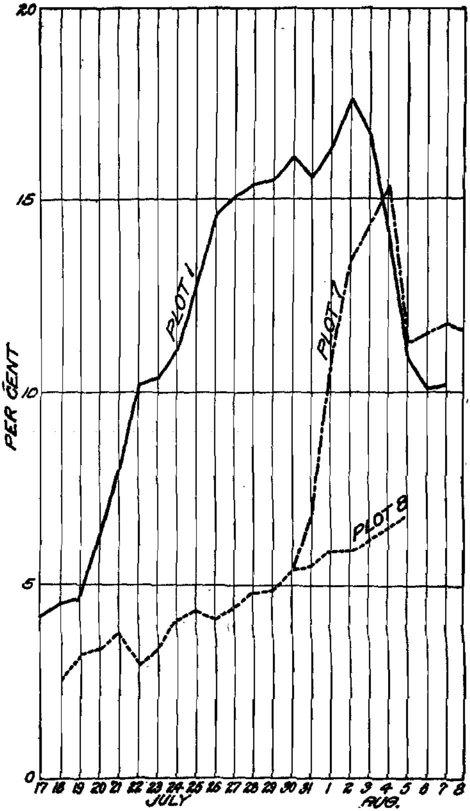


FIG. 2.—Percentage of ash, by progressive three-day averages, in the rachis of Hannchen barley grown on plots variously irrigated at Aberdeen, Idaho, in 1917.

the ash content of the soil is low, there was a much lower percentage of ash than in the western-grown samples. The analyses from the West, are, however, more significant in this connection, as shattering occurs much more commonly in the arid regions than in the humid regions.

In Table III it will be seen that the variety containing the highest percentage of ash in the rachis was an awnless variety. The second highest was a hooded sort. Among those varieties having an ash content over 9 per cent in the rachis were the awnless and hooded varieties referred to above, a variety from north Europe which was known to shatter badly, and C. I. No. 1449, a short-awned variety from Japan. In the original importation from which this last variety was obtained, there were two types of barley, differing only in the length of awn. C. I. 1449, which was short-awned, contained 10.1 per cent of ash in the rachis, while C. I. 1450, the long-awned strain, contained only 8 per cent.

The rachises of most of the common 2-rowed varieties are rather high in ash, many of them containing from 7 to 9 per cent when grown at Chico. The two samples of C. I. 1121 were taken from different parts of the nursery. The analyses show that there was very little variation due to location. C. I. No. 957, 973, and 972 are all of the Manchuria type. They contain less ash in their rachises than do most of the 2-rowed, but distinctly more than do the Coast types, C. I. No. 1076, 190, and 1074, which follow them in the table.

A number of smooth-awned varieties of hybrid origin are found in the table. These were included because of the potential economic importance of smooth-awned strains. The awn of the common barley is extremely harsh and is very objectionable to farmers and feeders. The annual acreage of barley is undoubtedly reduced because of the discomfort in handling the crop. On the other hand, it is known that the awn possesses a physiological function and it is improbable that maximum yields can be obtained from awnless and hooded varieties. In order to retain the functional value of the awn and at the same time to remove its objectionable features, the smooth-awned strains have been produced.

From the analyses given it appears that the smoothness of the awn has in no wise limited its function. One smooth-awned strain is included which has an ash content in the rachis of over 9 per cent. There are two strains with ash contents of nearly 8 per cent. Three others are found at the very bottom of the table with an ash content in the rachis of about 5 per cent. It is evident that in the latter varieties the low ash content is not due to any inactivity of the awn, as the awns themselves contain over 30 per cent of ash, indicating that they have been very active in transpiration. As can be seen in Table II, the Hannchen variety would come in the upper part of the list given in Table III.

## ASH OF THE PALEAS

Ash determinations were made on the paleas of the samples reported in Table I. These determinations are found in Table V. The ash content of the paleas is quite comparable with that of the awn as far as the nature of the daily deposits are concerned. While the total per-

centage at maturity is much less, there is the same uniform increment from flowering until maturity. As with the awns, the daily increase on plot 8, which received no irrigation after flowering, was less than on the other plots which received one or more irrigations. Unlike the case of the awn, however, the maximum percentage of ash was reached on plots which suffered to a considerable degree from lack of water. The ash content showed a response to irrigation even on plot 7.

TABLE V.—*Percentage of ash in the paleas of Hannchen barley from variously irrigated plots at Aberdeen, Idaho, in 1917*

| Date. | Plot 1. | Plot 2. | Plot 3. | Plot 4. | Plot 5. | Plot 6. | Plot 7. | Plot 8. |
|---|---|---|---|---|---|---|---|---|
| July 16 | 4.9 | 10.7 | ........ | ........ | ........ | ........ | ........ | 4.0 |
| 17 | 8.0 | 7.3 | ........ | ........ | ........ | ........ | ........ | 4.4 |
| 18 | 5.3 | ........ | 7.1 | ........ | ........ | ........ | ........ | 4.3 |
| 19 | 6.5 | 5.7 | 8.2 | ........ | ........ | ........ | ........ | 5.3 |
| 20 | 6.4 | 6.4 | 8.2 | 8.6 | ........ | ........ | ........ | 5.1 |
| 21 | 9.9 | 7.3 | 10.1 | 8.3 | ........ | ........ | ........ | 5.6 |
| 22 | 9.4 | 7.2 | 9.9 | 8.3 | ........ | ........ | ........ | 6.0 |
| 23 | 9.5 | 8.0 | 10.7 | 6.4 | 10.1 | ........ | ........ | 6.3 |
| 24 | 10.4 | 8.2 | 11.5 | 7.2 | 9.9 | ........ | ........ | 7.0 |
| 25 | 12.6 | 8.4 | 7.5 | 7.1 | 10.2 | ........ | ........ | 7.0 |
| 26 | 12.7 | 8.7 | 8.5 | 11.0 | 10.6 | 6.9 | ........ | 7.5 |
| 27 | 12.6 | 9.0 | 8.5 | ........ | 10.7 | 7.6 | ........ | 7.2 |
| 28 | 12.6 | 8.2 | 9.2 | ........ | 12.0 | 10.9 | ........ | 7.1 |
| 29 | 13.9 | 10.7 | 8.4 | ........ | 7.7 | 10.9 | 7.9 | 7.7 |
| 30 | 13.2 | 9.7 | 9.4 | 11.6 | 8.7 | 11.2 | 8.1 | 7.6 |
| 31 | 13.4 | ........ | 11.1 | 12.3 | 9.3 | 12.5 | 9.0 | 8.1 |
| Aug. 1 | 13.8 | 10.2 | 12.3 | 11.7 | 8.0 | 11.9 | 8.2 | 7.9 |
| 2 | 13.9 | 13.7 | 13.3 | 9.8 | 10.1 | 12.7 | 8.2 | 8.3 |
| 3 | 14.6 | 13.5 | 12.1 | 11.7 | 8.8 | 9.5 | 8.2 | 8.4 |
| 4 | 11.4 | 13.9 | 12.5 | 8.9 | 12.2 | 12.7 | 11.8 | 9.1 |
| 5 | 12.5 | ........ | 13.3 | 9.5 | 13.8 | 10.7 | 11.6 | 9.2 |
| 6 | 12.1 | 11.6 | 14.0 | 9.7 | 13.0 | 13.0 | 12.7 | 8.8 |
| 7 | 12.9 | 10.8 | 10.0 | 9.4 | 13.4 | 10.3 | 9.7 | ........ |
| 8 | 13.3 | 10.7 | 14.0 | 9.1 | 13.3 | 8.5 | 9.9 | ........ |
| 9 | 16.2 | 10.7 | 15.3 | 15.4 | 12.7 | 11.7 | 9.6 | ........ |

No determinations were made which would show the variations in the ash content of the paleas of different varieties. With mature samples, such as those discussed in Table III, it is impossible to strip the paleas from the kernels. For the same reason the analyses of the kernels in Table III are not particularly valuable. The ash content of the caryopsis is much lower than that of the inclosing glumes, so that any variations in the ash of the glumes, or in the proportion of caryopsis to glumes, appear in the table as a difference of the ash content of the kernels.

## ASH IN THE KERNELS

In the previous papers published on kernel development,[1] the ash in the kernel was computed as a percentage of the dry matter. In the case

[1] HARLAN, Harry V. DAILY DEVELOPMENT OF KERNELS OF HANNCHEN BARLEY FROM FLOWERING TO MATURITY AT ABERDEEN, IDAHO. *In* Jour. Agr. Research, v. 19, no. 9, p. 393–430, 17 fig., pl. 83–91. 1920. Literature cited, p. 429.

HARLAN, Harry V., and ANTHONY, Stephen. OP. CIT.

of the awns, paleas, and rachises, this is probably the best method of comparison. These organs do not increase perceptibly in size during the time the deposit of ash is taking place. In the awns the deposit probably consists of ash eliminated from the cell sap. In consequence of this very heavy deposit, the ash in the awn reaches a percentage of the

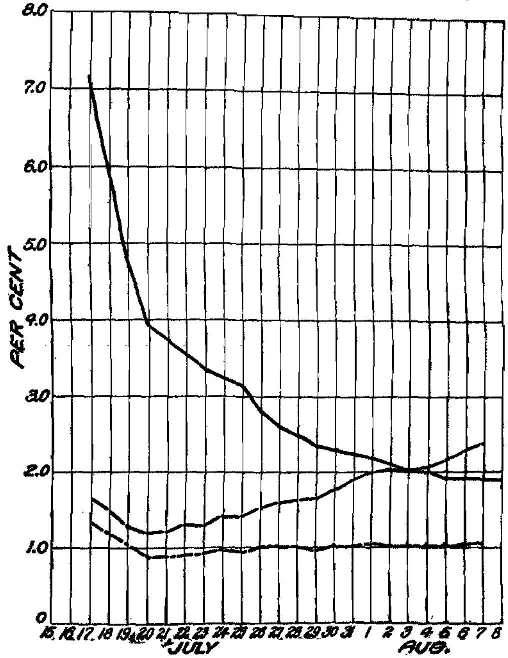


FIG. 3.—Percentage of ash in barley kernels, computed on the basis of dry matter (solid line), water (broken line), and wet weight (dots and dashes), from flowering to maturity, at Aberdeen, Idaho, 1917.

total weight which overshadows any variation of sample or defect of method in calculation. In the case of the kernel it is not thought that the dry matter is a desirable basis of computing ash. When computed on this basis, as will be seen in figure 3, the ash content at flowering time is very high. In most determinations it has been around 8 per cent at this period of growth.

Shortly after fertilization the percentage of ash commences to drop, falling very rapidly for a few days and then more gradually until complete maturity. This is obviously not a clear statement of what occurs. The percentage of ash on the dry-matter basis is a perfectly accurate statement, but the plotted curve of such percentage does not give a graphic idea of what is taking place in the kernel. There is a daily increase in total ash. This increase is almost uniform. The total ash content of the kernel when plotted is an ascending nearly straight line. Whether more of this ash is contained in one part of the kernel than another is not apparent. The ash at flowering time must be in solution and in the protoplasm. There has not been time for any deposit in the newly formed cell walls. Until several days after flowering the ash content must be in the cell sap, the proteids, and such penetration of cell walls as probably would occur if the tissue were not living.

As about 80 per cent of the content of a newly formed kernel is water, it was thought at first that calculating the percentage of ash on the basis of water would be the best method of comparison. In the very early stages, before any deposit could occur in the cell walls, this might be true. However, as the development of the kernel proceeds, the water occupies a smaller and smaller percentage of the kernel. Not only does the proportion of cell walls increase, but the proportion of the proteid matter in the active tissue probably is increased by the growth of starch grains. These starch grains, being formed in the cells, must occupy space previously largely occupied by cell sap.

If the ash is to be accounted for entirely on the basis of cell sap, the concentration of the cell sap must show a progressive increase to account for the total ash. This is highly improbable. The curve of percentage of ash based on water content is, however, more regular than the one based on dry matter and is in the direction of the actual ash deposit.

The ash was finally computed on the basis of the wet weight of the growing kernel. By computing it on this basis, allowance was made for both the ash in the cell sap and that in the organized components of the cell. The use of such a method assumes that the ash in the dry matter would be a mechanical infiltration from the cell sap which would eventually show the same percentage throughout the cell. When computed in this way a striking uniformity is revealed (fig. 3). Although the proportion of water and dry matter varies over a range of 40 per cent during the growing period, the percentage of ash on the basis of wet weight is almost constant. In Table VI are given the analyses of kernels from various plots. These plots differ in irrigation, in the years grown, and in the variety used. The awns from the same samples from which the kernels were taken show a variation of 15 per cent under the radical changes of conditions of growth. The variation in the percentage of ash on the basis of wet weight of kernel is a matter of tenths of a per cent. Many of the apparent fluctuations have plausible explanations. At

Minnesota, for instance, the grain was badly lodged and ripened very unevenly. There was also considerable rain at ripening time which delayed the ripening of part of the spikes. That many of these irregularities were due to the stage of ripening was apparent in a table published in a previous paper.[1] In this table the kernels with high ash content are the kernels which weighed less than 50 mgm. In other words, they were kernels in which maturation had been carried to the point where the mechanical loss of water had reduced the wet weight below 50 mgm.

TABLE VI.—*Percentage of ash in kernels of barley from flowering to maturity, compile on the basis of the wet weight*

| Days from flowering. | Plot 1, 1917. | Plot 3, 1917. | Plot 4, 1917. | Plot 5, 1917. | Plot 6, 1917. | Plot 7, 1917. | Plot 8, 1917. | Hannchen, 1916. | Hannchen, clipped, 1916. | Manchuria, 1915. | Manchuria, clipped, 1915. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 0.95 | 1.16 |
| 1 | 1.41 | ..... | ..... | ..... | ..... | ..... | 2.23 | 0.62 | 1.18 | 1.02 | 1.07 |
| 2 | 1.39 | 1.31 | ..... | ..... | ..... | ..... | .09 | .81 | .79 | .90 | .95 |
| 3 | 1.17 | .88 | ..... | ..... | ..... | ..... | 1.61 | 1.04 | ..... | .84 | .81 |
| 4 | .90 | .87 | ..... | ..... | ..... | ..... | 1.07 | .93 | .92 | .96 | .96 |
| 5 | .91 | .76 | 0.96 | ..... | ..... | ..... | .96 | .85 | .96 | .81 | .95 |
| 6 | .82 | 1.14 | 1.18 | ..... | ..... | ..... | .80 | .88 | .93 | .89 | .93 |
| 7 | .90 | .85 | .94 | ..... | ..... | ..... | .94 | ..... | ..... | .77 | .98 |
| 8 | 1.03 | 1.00 | .94 | 1.11 | ..... | ..... | .91 | .87 | .98 | .91 | .95 |
| 9 | .90 | 1.14 | .97 | .87 | ..... | ..... | .73 | 1.00 | .96 | 1.02 | 1.04 |
| 10 | .94 | 1.21 | 1.27 | .79 | ..... | ..... | 1.03 | 1.14 | .94 | 1.08 | 1.07 |
| 11 | .97 | 1.55 | 1.12 | .78 | 0.81 | ..... | .97 | 1.04 | 1.06 | ..... | ..... |
| 12 | 1.01 | .82 | 1.00 | 1.12 | .86 | ..... | .91 | 1.04 | 1.15 | 1.12 | 1.03 |
| 13 | .97 | .95 | 1.07 | ..... | .81 | ..... | 1.06 | 1.02 | 1.15 | 1.13 | 1.22 |
| 14 | 1.01 | .92 | 1.25 | .95 | .76 | 0.85 | 1.00 | ..... | ..... | 1.13 | 1.01 |
| 15 | 1.01 | ..... | .95 | 1.08 | .78 | .96 | 1.14 | 1.06 | 1.17 | 1.13 | 1.14 |
| 16 | 1.06 | 1.04 | 1.00 | .96 | 1.08 | .86 | 1.04 | 1.22 | 1.20 | 1.12 | 1.15 |
| 17 | 1.10 | .96 | .94 | 1.04 | .90 | 1.01 | 1.00 | 1.08 | 1.32 | 1.17 | 1.26 |
| 18 | .99 | .98 | 1.10 | 1.06 | .92 | .94 | 1.06 | 1.34 | 1.26 | ..... | ..... |
| 19 | 1.02 | 1.06 | .99 | 1.04 | .82 | .85 | .91 | 1.19 | 1.26 | 1.16 | 1.25 |
| 20 | 1.01 | 1.04 | 1.11 | 1.03 | 1.49 | .84 | .89 | 1.19 | 1.31 | 1.11 | 1.28 |
| 21 | 1.02 | 1.02 | .97 | 1.12 | 1.15 | .88 | 1.17 | ..... | ..... | 1.29 | 1.44 |
| 22 | 1.04 | 1.08 | 1.36 | .91 | 1.32 | 1.18 | 1.23 | 1.35 | 1.21 | 1.26 | 1.44 |
| 23 | 1.08 | 1.08 | 1.14 | 1.40 | .90 | 1.15 | ..... | 1.34 | 1.42 | 1.20 | 1.28 |
| 24 | 1.08 | 1.00 | 1.00 | 1.06 | .93 | .86 | ..... | 1.51 | 1.57 | 1.25 | 1.44 |
| 25 | 1.07 | ..... | ..... | 1.04 | ..... | ..... | ..... | 1.62 | 1.75 | ..... | ..... |
| | | | | | | | | | | 1.25 | 1.20 |
| | | | | | | | | | | 1.02 | 1.37 |
| | | | | | | | | | | 1.08 | 1.30 |
| | | | | | | | | | | 1.41 | 1.26 |
| | | | | | | | | | | 1.79 | 1.71 |

At final maturity, where the base of calculation was reduced by the rapid mechanical loss of water, there was sudden rise in the percentage of ash. This increase is taken to indicate maturity. The taking of samples usually ceased just before the final rapid fall of water content. The glumes began to adhere to the caryopsis several days before maturity. After they commenced to adhere the separation of glumes and caryopsis

[1] HARLAN, Harry V., and ANTHONY, Stephen. OP. CIT.

was imperfect. Fragments of the inner tissues of the glumes frequently remained clinging to the caryopsis and pieces of the outer layers of the pericarp were as often removed with the glumes. It was thought that this small interchange of tissue did not affect the results, but to be certain a comparable series of kernels from a naked barley was studied. The results were added to figure 4. The curve of the percentage of ash based on wet weight is essentially the same as in the hulled varieties. In this figure it is apparent that neither the application of irrigation water nor the difference in the character of the barley influenced the percentage of ash when computed on the basis of wet weight. The analyses of a number of mature samples of commercial naked varieties were also available. When the ash was recalculated on a wet basis of 45 per cent water the ash content was about the same as that obtained in the field.

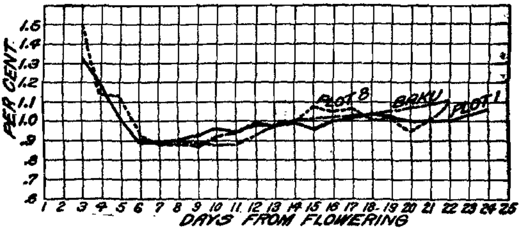


FIG. 4.—Graph showing percentage of ash on the basis of wet weight in the kernels of Hannchen barley on 2 plots differently irrigated, in 1917, and of Baku barley grown in another year at Aberdeen, Idaho.

## DISCUSSION OF RESULTS

The extremely heavy deposit of ash in the awns of barley indicates that the awn, or parts of the awn, are used as a depository for the excess ash absorbed by the roots. The fact that some varieties contain much more ash in the awns and rachises than others is due probably to two causes. There most probably is a difference between varieties in the amount of water transpired. As was shown in the irrigation plots at Aberdeen, this results in a marked variation of ash deposit. There may also be a difference in the selective functions of the roots of different varieties. Some varieties may absorb more ash from the soil than do others. This is strongly indicated in the ash content of the rachises. It is also of greatest importance in this connection. Varieties of the Coast type are characterized by a low ash content of the rachis. In most of the shattering varieties the rachises are high in ash content. The hooded varieties have long been known to shatter badly. From results previously reported it would seem that much of this is due to the loss of the awn as an organ partially utilized for the elimination of ash. On the other hand,

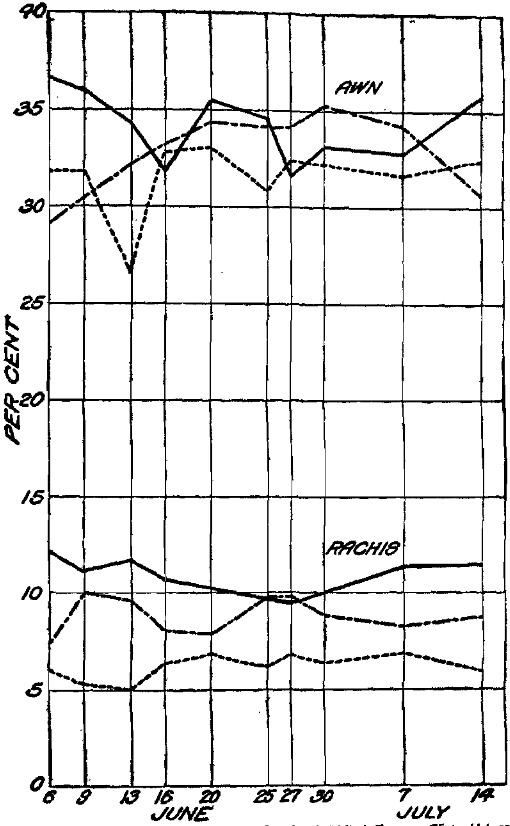


FIG. 5.—Percentage of ash in the awn and rachis of Hannchen (solid line), Tennessee Winter (dots and dashes), and Coast (broken line) barleys sampled on 10 different dates, at Chico, *Calif.*, in 1917.

the hooded varieties most largely grown have come from hybrids whose parents were both from humid districts. The resulting hybrids might be less brittle if parents adapted to arid conditions were used.

On account of the low ash content of the rachis of the Coast barley (fig. 5), varieties of this type may be useful in the production of non-shattering awnless and hooded sorts. Barleys of the Coast group probably take less ash from the soil than do most others. There certainly is less deposited in the awns and rachises than in those of the other common varieties. Crosses of hooded varieties with varieties of the Coast type should give hooded segregates which are less brittle than the common hooded forms. Indeed the Meloy, one of the best hooded varieties under cultivation, is probably the result of such a cross. In a more complex cross it might be possible to use some of the characters of the Hanna variety. The Hanna is not classified as a shattering variety, yet its rachis contains a high percentage of ash. In this case the rachis is able to withstand a heavy deposit. It is possible that this resistance might also be of use, although hooded crosses of this sort, when not combined with the Coast, have not been very promising. In this connection it is desired not to overemphasize the relation of ash content to shattering. There is an obvious relation, but the ash content is only one of a number of factors. The tenacity of the vascular bundles, the character of the cell walls, and the size of the rachis, all have a bearing on shattering. There is also more than one type of shattering. In the Manchuria barley, for instance, when grown in Idaho under irrigation, the kernels become loosened from the spike without the rachis itself being affected. In this case the ash content of the paleas may have some bearing on deciduousness.

The ash of the kernel is of particular interest. In this case all of the ash is contained within cells which are engaged in highly active metabolism. The ash is either in the cell sap itself, the active proteid content, or the cell walls. When the ash is computed on total wet weight a very uniform percentage is maintained. It is obvious that at no time is any part of the kernel set aside as a repository for ash. There is very little difference between the kernels of plants which are dying from drouth and those which are growing under an ample supply of water. Why the ash content of the active kernel is maintained at a nearly constant precentage and whether a higher percentage of ash than that exhibited interferes with normal metabolism is not indicated from these analyses. That the uniform percentage of ash in some way is connected with the fundamental processes of growth is indicated further by the fact that the percentage coincides with that found in roots, tubers and fruits, all storage organs, and even with that of meat and eggs.

The percentage of ash based on the wet weight of kernel is not quite constant. There is a loss in percentage immediately following fertilization and then a gradual increase until full maturity. This behavior can not be adequately interpreted. It appears that at the time of fertilization the ash content of the ovary is very high. Immediately after fertilization there is a decided distention, partially due to the turgidity of

a high water content. The tissues arising from the fertilized egg cell occupy a very small part of the growing kernel for several days after fertilization. The ovary wall increases very rapidly. A tissue develops at the end of the kernel arising from the ovary walls which persists for a considerable time and which grows very rapidly for the first few days after flowering. Histological sections of this tissue indicate that very little is concerned in its growth except the addition of cell walls, the enlarging of cells, and the increase of the watery cell content. A small starch deposit is found in the cells, but it is negligible. This high proportion of watery tissue might result in the drop of ash content immediately following fertilization. The gradual increase from then to maturity may be due to the fact that the proteids contain a greater percentage of ash than does the cell sap, or it may come about from a light deposit in some limited tissue of the caryopsis.

## SUMMARY


The awn of barley receives a very large deposit of ash, comprising over 30 per cent of the dry weight in some varieties. Barleys differ in the amount of ash deposited in the awn and probably in the selective function of the absorbing roots. Within a variety the amount of ash in the awn is correlated with the supply of soil water and probably with the amount of water transpired.

There are varietal differences in the amount of ash deposited in the rachis. The rachises of hooded and awnless varieties are usually high in ash and usually brittle. The tendency to shatter may possibly be overcome in hooded varieties by crossing them with barleys of the Coast type, which have little ash in their rachises.

No part of the kernel proper is used as a repository for ash. The ash of the kernel is the ash of cell sap and of highly active protoplasm. When computed on the basis of the wet weight, the wet weight being a measure of the organ when active, there is almost no variation in the proportion of ash. During most of the period of growth the variation is only 0.3 of 1 per cent, the content increasing gradually from slightly less than 1 per cent in early growth to slightly more than 1 per cent at maturity.

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## TEMPERATURE RELATIONS OF STONE FRUIT FUNGI

By CHARLES BROOKS and J. S. COOLEY, *Pathologists, Fruit Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

The two fungi that cause the heaviest market losses on peaches and other stone fruits are *Sclerotinia cinerea* (Bon.) Wor. and *Rhizopus nigricans* Ehr. The former is often referred to under its conidial name of Monilia and is the cause of brown rot, while the latter is the cause of black mold rot.

The present paper gives the results of investigations in regard to the temperature responses of these two fungi under various conditions of growth.

In all the experiments except that reported in figure 1 the spores were inoculated into the fruit from pure cultures. Except where otherwise stated the cultures were obtained from the host into which the inoculations were made. The fruit was warm when inoculated but after inoculation was placed in moist chambers and stored at once at the temperature indicated. Five or more peaches or prunes were used at each temperature in each test. The fruit was at the proper maturity for picking and shipping and was carefully selected for quality and soundness. In dividing the fruit into lots for distribution at the various temperatures uniformity was secured by selecting seven peaches or prunes (or as many as there were temperatures) that were similar in size, color and degree of maturity and distributing these one each in seven moist chambers and repeating the process till the desired amount of fruit was obtained.

Records were made of the diameters of the rots at intervals of one or two days, and the average of all the rots at a given temperature on a particular date was taken as a basis for plotting the curves in the accompanying figures. The equipment used in securing the various temperatures has been described in an earlier publication.[1]

### SWEET CHERRIES

In 1919 a temperature experiment was made on Governor Wood cherries. These had been shipped by express from Wallingford, Conn., to Washington, D. C., and arrived somewhat bruised and with considerable brown rot. The specked and rotten cherries were discarded, but the slightly bruised ones were included in the experiment. The cherries were divided into five equal lots and distributed without inoculation at

[1] BROOKS, Charles, and COOLEY, J. S. TEMPERATURE RELATIONS OF APPLE-ROT FUNGI. *In* Jour. Agr. Research, v. 8, no. 4, p. 139–164, 25 fig., pl. 1–3. 1917.

five different temperatures.[1] After 10 days' storage notes were taken and results obtained as shown in figure 1.

All of the cherries at 15° and 20° C. were partially or entirely rotten and nearly all of those at 10°. At 5° sixty-six per cent were affected, and at 0° thirty-four per cent. The results show the great inhibiting effect of low temperatures but perhaps give greater emphasis to the extreme difficulty of controlling Monilia rot at any temperature when the fruit has already received bad treatment and an opportunity has been given for the rot to pass through its initial stages while the fruit was warm.

## PRUNES

But one temperature test has been made on prunes. The fruit was from Wenatchee, Wash., and was shipped from that point in a pony refrigerator August 31, 1920, arriving in Washington, D. C., in good condition 13 days later. Inoculations were made with Monilia and Rhizopus, and the fruit was distributed at once to the various temperatures. Figure 2 shows the development of the rots 5 days after inoculation.

## PEACHES

A large number of temperature experiments have been made with Monilia and Rhizopus on peaches. The Carman, Belle, and Elberta peaches used in the 1918 experiments were purchased in the Washington market. The Belle and Elberta used in 1919 were from Rockville, Md., and the experiment was started the day after they were picked. The Carman and Belle peaches used in 1920 were from Vienna, Va., and were inoculated the day after they were picked. These peaches were slightly greener than those of the other experiments.

The curves of the various figures show very great uniformity. The Rhizopus cultures from peaches gave results similar to the cultures from cherries and strawberries, both in temperature response and in rapidity of rotting.

An interesting contrast is seen between the behavior of the fungi on peaches and on dextrose potato agar. A comparison of figures 3, 4, 5, and 6 with figure 7 shows that Monilia has grown just as freely at the higher temperatures and much earlier and more rapidly at the lower temperatures when grown on peaches than when on agar. At 10° C. rots usually became evident on the fruit within 3 days, while on the agar there was practically no growth at the end of 7 days. At 5° the rots were well started in 6 days, while the agar colony had scarcely made an equivalent growth at the end of 14 days. At 2½° the rots made a start in 8 to 12 days, but there was no evidence of growth on the agar at the end of 20 days. A comparison of figures 8, 10, 12, 14, 15, 16, and 17 with figures 9, 11, and 13 shows that the reverse condition holds with Rhizopus. This fungus made a more rapid growth and developed at lower temperatures on the culture media than it did on the fruit. On both food materials it had its most rapid growth at 30°. With the culture media the growth at 20° and 25° was but little slower than at 30°, but on the peaches the growth at 20° fell far behind that at 30°. At 15° and also at 10° the

[1] Temperature equivalents:

| °C. | °F. | °C. | °F. |
|---|---|---|---|
| 20 | 68 | 5 | 41 |
| 15 | 59 | 0 | 32 |
| 10 | 50 | .. | .. |

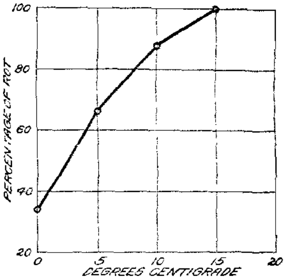


FIG. 1.—Natural infections of brown rot on Governor Wood cherries. The base line shows the temperatures and the perpendicular the percentage of cherries affected with brown rot.

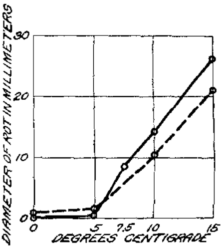


FIG. 2.—Monilia (solid line) and Rhizopus (broken line) on Italian prunes. Temperature is indicated on the base line and diameter of rot on the perpendicular.

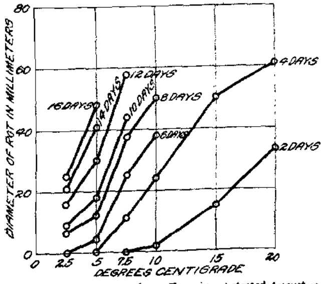


FIG. 3.—Monilia on Elberta peaches. Experiment started August 20, 1919.

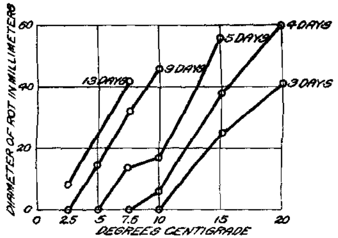


FIG. 4.—Monilia on Belle peaches. Experiment started August 27, 1919.

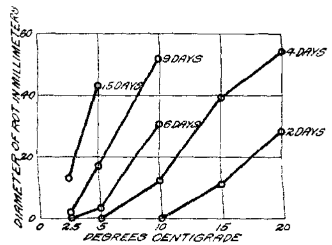


FIG. 5.—Monilia on Carman peaches. Experiment started August 5, 1920.

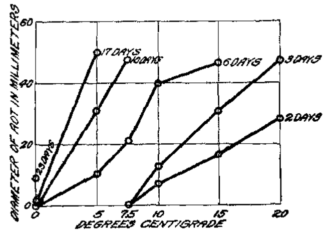


FIG. 6.—Monilia on Belle peaches. Experiment started August 28, 1920.

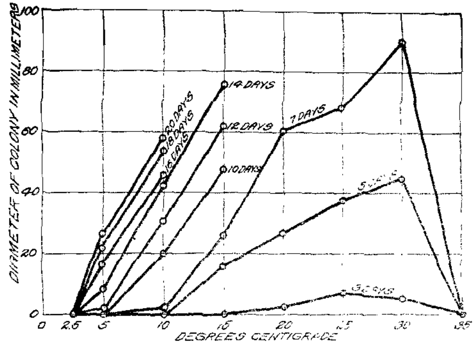


FIG. 7.—Peach Monilia on potato agar with 2 per cent dextrose added. In Petri plates. Experiment started November 22, 1918.

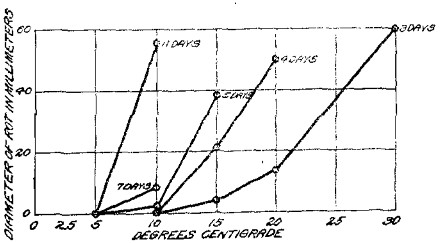


FIG. 8.—Peach Rhizopus on Elberta peaches. Experiment started August 23, 1918.

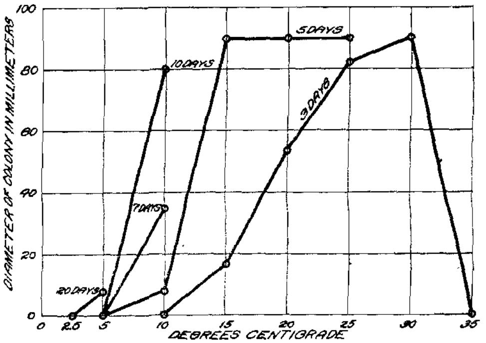


FIG. 9.—Peach Rhizopus on potato agar with 2 per cent dextrose added. Experiment started November 22, 1918.

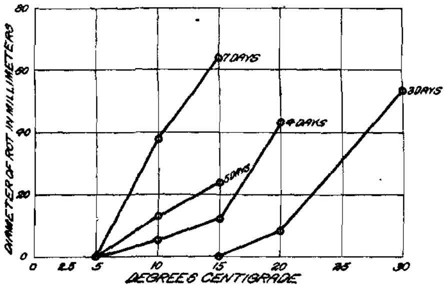


FIG. 10.—Cherry Rhizopus on Elberta peaches. Experiment started August 23, 1918.

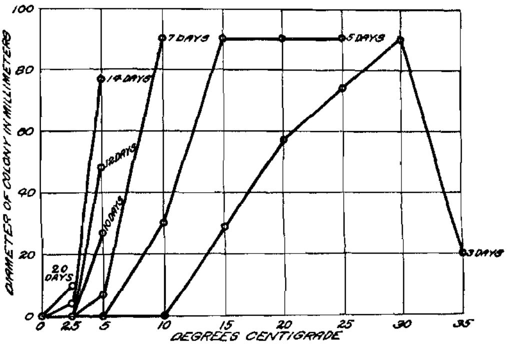


FIG. 11.—Cherry Rhizopus on potato agar with 2 per cent dextrose added. Experiment started November 22, 1918.

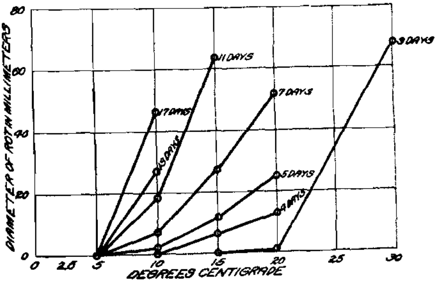


FIG. 12.—Strawberry Rhizopus on Elberta peaches. Experiment started August 23, 1918

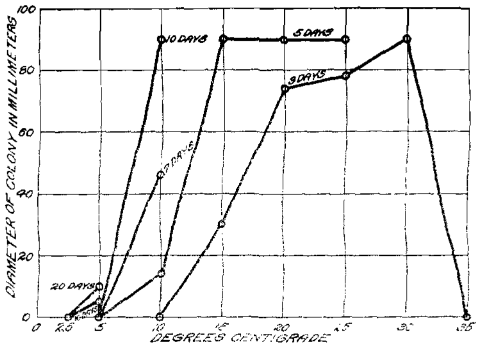


FIG. 13.—Strawberry Rhizopus on potato agar with 2 per cent dextrose added. Experiment started November 22, 1918.

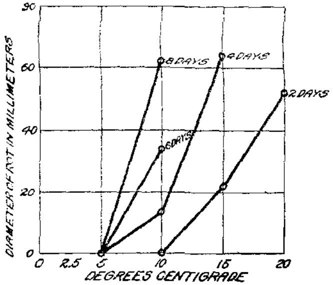


FIG. 14.—Peach Rhizopus on Carman peaches. Experiment started August 1, 1918.

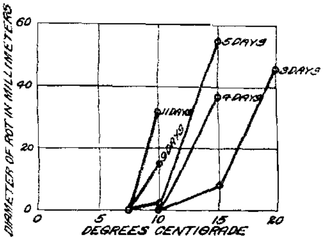


FIG. 15.—Peach Rhizopus on Belle peaches. Experiment started August 27 1919.

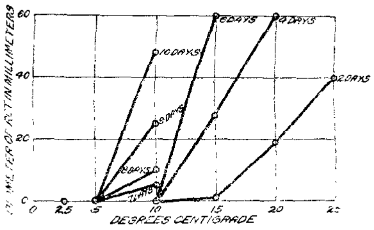


FIG. 16.—Peach Rhizopus on Carman peaches. Experiment started August 5, 1920.

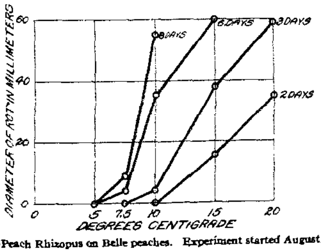


FIG. 17.—Peach Rhizopus on Belle peaches. Experiment started August 28, 1920.

growth started earlier and proceeded more rapidly on the agar than on the peaches. The fungus finally made a slow growth on the agar at 5°, but on peaches it made no growth at this temperature and with the exception of a few overripe peaches none at 7½°.

These contrasts in the behavior of the two fungi can probably be partly explained by the fact that Monilia is a parasite and adapted to growth on living material, while Rhizopus is a saprophyte and suited to growth on dead material, like the agar or inactive living material such as overripe fruit. It is interesting to note that with both fungi unfavorable food material and unfavorable temperatures work together in delaying growth, one unfavorable factor adding to the other in delaying or inhibiting activity.

A study of figures 3, 4, 5, 6, 18, and 20 gives a detailed idea of what can be expected of Monilia rot at any transportation or storage temperature. The results in the last two figures have been obtained by averaging those of the first four figures and therefore stand as a summary of the various experiments. With fruit that is infected with brown rot (Monilia) 3 days at 15° C. would result in heavy losses, 3 days at 10° would mean badly specked fruit that would go down rapidly at that temperature and that would be entirely destroyed by a day at a higher temperature. Brown rot can not get started in 3 days' time at 7½°, but by the end of the fourth day fruit at that temperature may be specked with rot. In 6 days the fruit at 5° may be spotted, in 9 to 12 days growth may be evident at 2½°, and at the end of 3 weeks rots may have started at 0°. Brown rot does not develop rapidly at the lower temperatures even when well started, yet its later growth is inhibited far less than its initial stages.

A study of figures 8, 10, 12, 14, 15, 16, 17, 19, and 21 shows that Rhizopus has more decided temperature limitations than those that have been pointed out for Monilia. The results in the last two figures have been obtained by averaging those of the first seven and therefore stand as a summary of the various Rhizopus experiments. At 15° and 20° C. the growth rate of Rhizopus rot is practically the same as that of Monilia rot, Rhizopus being a trifle more rapid at 20° and Monilia just a little more rapid at 15°. At 10° Monilia rot develops more than twice as fast as Rhizopus rot, and at 7½° Rhizopus is practically eliminated.

Whether Rhizopus could make any start whatever at 7½° C. seemed to be determined mainly by the maturity of the fruit. The curves of figure 15 show that Rhizopus had not made a start at 7½° in 11 days. The peaches at that temperature were still free from rot at the end of 14 days, were removed to a warm room at that time, and were entirely rotted with Rhizopus 2 days later. The results show that the fungus was held completely in check at 7½° but was alive and ready for rapid development when given a more favorable temperature. With the experiments reported in figure 17, Rhizopus had produced evident rotting at 7½° in 6 days. At that time only the ripest peaches were affected, but at the end of 12 days rots began to develop on the greener peaches. When once started at this temperature Rhizopus rot made a fairly rapid growth. The results as a whole show that with the usual number of days in transit for most peach shipments Rhizopus can produce little or no damage at 10° and none at 7½°.

Figures 3 to 21, inclusive, show the development of the rots when the fruit is stored at the given temperatures immediately after inoculation. Figures 22, 23, and 24 show the effect of 1 day's delay at a higher tem-

perature than that at which the fruit was finally held. A study of the figures brings out the facts that with Monilia 1 day at 25° C. followed by 1 day at 10° results in as large spots as 5 days at 10°; 1 day at 20° followed by 1 at 10° results in as large spots as 4 days at 10°; 1 day at 25° followed by 1 at 7½°, in as large spots as 6 days at 7½°; 1 day at

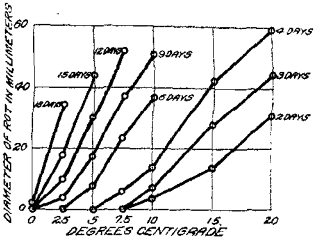


FIG. 18.—Monilia on peaches. A summary of the experiments on brown rot obtained by averaging the percentages of figures 3, 4, 5, and 6.

25° followed by 1 day at 5°, in as large spots as 10 days at 5°; 1 day at 25° followed by 1 day at 2½°, in as large spots as 12 days at 2½°. It will also be seen that with Rhizopus 1 day at 25° followed by 1 day at 10°, or 1 day at 15° followed by 4 at 10°, results in larger spots than 7 days continuously at 10°.

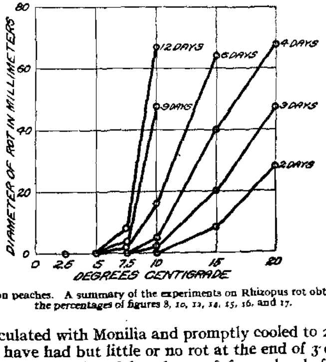


FIG. 19.—Rhizopus on peaches. A summary of the experiments on Rhizopus rot obtained by averaging the percentages of figures 8, 10, 12, 14, 15, 16, and 17.

Peaches inoculated with Monilia and promptly cooled to 2½°, 5°, 7½°, or even 10° C. have had but little or no rot at the end of 3 or 4 days, but similarly inoculated peaches delayed at 25° for 1 day before storing at these lower temperatures have developed so much rot by the end of the fourth day after inoculation that they were commercially worthless. Peaches inoculated with Rhizopus and promptly cooled to 10° have been

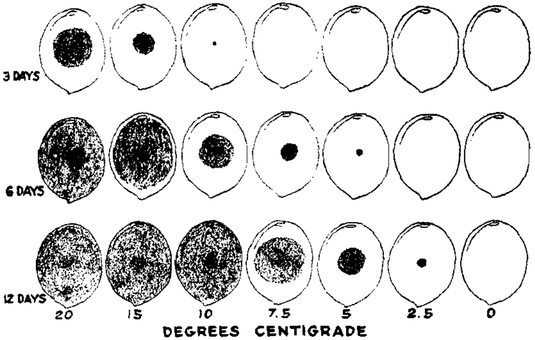


FIG. 20.—Monilia on peaches. The drawings represent the average of the results from the various experiments. The shaded portions indicate the extent of the decay. The upper series shows the size of the rots at the various temperatures after 3 days, the second series the size after 6 days, and the third the size after 12 days.

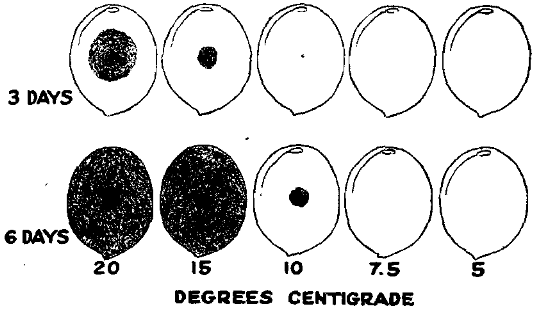


FIG. 21.—Rhizopus on peaches. The drawings represent the average of the results from the various experiments. The shaded portions indicate the extent of the decay. The upper series shows the size of the rots at the various temperatures after three days and the second series the size after six days.

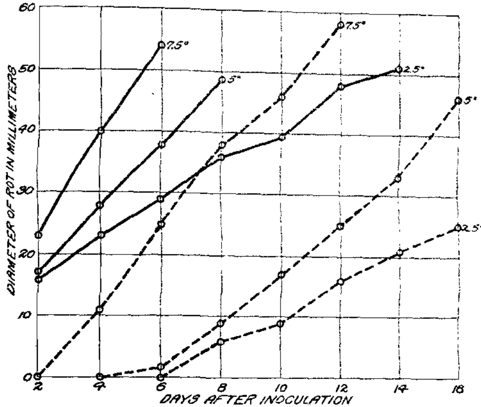


FIG. 22.—Effect on Monilia rot of one day's delay at 25° C. The base line shows the number of days after inoculation and the perpendicular the diameter of the rots. The curves show the rate of development of the rots at the temperatures indicated at the end of the lines. The dotted lines give the results on the fruit placed at once at the given temperatures and the solid lines the results on similar fruit delayed one day at 25° before placing at these temperatures. Elberta peaches, August 20, 1919.

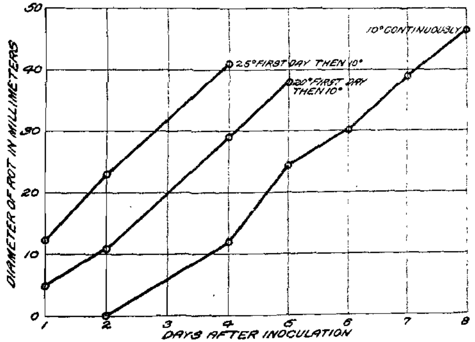


FIG. 23.—Effects on Monilia rot of one day's delay at 25° or 20° C. before storing at 10°. The base line gives the number of days after inoculation and the perpendicular the diameter of the rots. The curves show the development of the rots under the particular storage treatment. Experiment on Carman peaches, August 5, 1920.

entirely free from rot at the end of 6 days, while similarly inoculated peaches delayed for 1 day at 25° before storing at 10° have been almost entirely rotten at the end or 6 days, and those held at 15° before storing at 10° have been considerably damaged by the end of 6 days.

*The results show the great value of low temperatures in controlling peach rots and the extreme importance of securing these temperatures promptly. It is evident that in unfavorable weather success with long-distance shipments requires not only a low car temperature upon arrival at destination but a low temperature from the time the peaches are packed* and as much coolness as possible from the time they are picked. It is not an unusual thing for peaches to remain at the prevailing seasonal temperature for a day or more before being loaded into

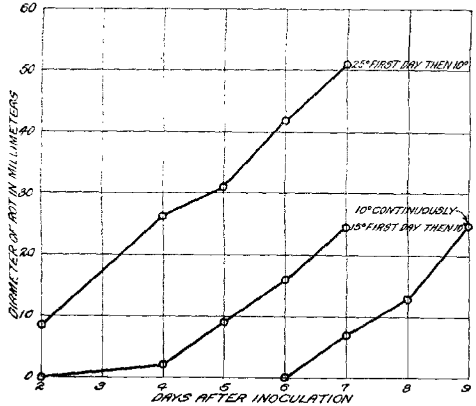


FIG. 24.—Effects on Rhizopus rot of one day's delay at 25° or 15° C. before storing at 10°. The base line gives the number of days after inoculation and the perpendicular the diameter of the rots. The curves show the development of the rots under the particular storage treatment.

the car, and with most makes of refrigerator cars and the usual methods of icing it is likely to be one or two more days before a really protecting temperature is secured. The fruit in the top layers is often still above 10° C. after several days in transit. Prompt loading, better refrigerator cars and heavier icing, particularly during the first part of a trip, would contribute greatly to lengthening the life of stone fruits; but the experiments that have been reported show that there would still be a gap that would sometimes result in heavy losses of fruit and that could only be filled by some method of precooling.

One of the unfortunate things in regard to delayed cooling is that its harmful effects may not be immediately evident. Peaches may appear practically sound after a delay before loading and cooling and yet that delay may have allowed the rots to make a start that will require

hat the temperature be held several degrees lower or that the destination be selected several days nearer in order to insure the delivery of sound fruit.

SUMMARY

(1) A temperature of 10° C. (50° F.) has held Monilia in check for one or two days and Rhizopus in check for three days. A temperature of 7½° C. (45½° F.) has held Monilia in check for three days and Rizopus in check for six or more days. A temperature of 5° C. (41° F.) has held Monilia entirely in check for four days, and 2½° C. (36½° F.) has held it in check for six days.

(2) Low temperatures have resulted in relatively less inhibition of growth with Monilia when grown on peaches than when grown on potato-dextrose agar, and a relatively greater inhibition with Rhizopus when grown on peaches than when grown on potato-dextrose agar. Both fungi have grown at lower temperatures on ripe fruit than on green fruit.

(3) Peaches stored at 10° C. (50° F.) immediately after inoculation have been three to five days slower in developing rot than those delayed one day at 25° C. (77° F.) before storing at 10° C. (50° F.). Peaches stored immediately at 7½° C. (45½° F.) have been five days slower in developing brown rot than those delayed one day at 25° C. (77° F. before storing at 7½° C. (45½° F.).

# TRANSPORTATION ROTS OF STONE FRUITS AS INFLUENCED BY ORCHARD SPRAYING

By Charles Brooks and D. F. Fisher, *Pathologists, Fruit Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

The present paper reports the pathological results of five years' shipping and storage experiments with green prunes and sweet cherries and is an attempt to demonstrate certain underlying facts that help to place the responsibility for transportation and market losses in perishable fruit shipments. Sprayed fruit and dusted fruit have been compared with untreated fruit from the same orchards under various transportation and storage conditions.

Spoilage of fruit has been almost entirely due to Monilia or brown rot [*Sclerotinia cinerea* (Bon.) Wor.], blue mold rot [*Penicillium expansum* (Lk.) Thom], and black mold rot [*Rhizopus nigricans* Ehr.]. Monilia attacks the fruit both in the orchard and on the market, but Penicillium and Rhizopus are able to develop only on the harvested fruit.

## SPRAYED AND UNSPRAYED SWEET CHERRIES IN TRANSIT AND IN STORAGE

The shipping experiments on cherries were made from the orchard of L. T. Reynolds of Salem, Oreg. The varieties used were Napoleon (Royal Ann), Black Republican, and Lambert. Various standard spray materials were used on the different orchard plots, including 2-4-50 Bordeaux plus 2 pounds of rosin fish-oil soap, 8-8-50 self-boiled lime-sulphur plus 2 pounds of rosin fish-oil soap, and commercial lime sulphur diluted 1 to 50. In the 1919 experiments a neutral Bordeaux (4 pounds copper sulphate in 100 gallons water neutralized with lime) was substituted for the 2-4-50 Bordeaux, a casein spreader [1] was added to the lime sulphur solution, and one plot was treated with 85-0-15 [2] sulphur dust.

In 1915 sprayings were made May 7 and 8 and June 1; in 1916, April 1, April 21, May 12, and June 15; in 1917, April 25, May 14, May 31, and June 22; in 1918, April 15, May 2, May 17, and June 18; and in 1919, June 7 and June 16. The earlier applications (before May 10) were for the control of blossom infection and probably had little effect upon the occurrence of rot on the ripe fruit.

In 1915 and 1916 there was practically no foliage injury from any of the spray materials used, but in each of the following three years very definite injury occurred on particular plots. In 1917 and 1918 lime sulphur caused heavy defoliation, and in 1919 Bordeaux produced considerable foliage injury. All of the spray materials, with the possible exception of neutral Bordeaux, reduced the size of the cherries. In most years this was scarcely perceptible, but in 1917 the dwarfing effect was sufficient to cause considerable loss.

The cherries of the Willamette Valley are often seriously damaged with brown rot, yet during the five years' work at Salem there was not a season in which the experimental orchard had as much as 1 per cent of rot at picking time on either the sprayed or unsprayed fruit.

Shipping experiments were made each year to determine the effect of the orchard treatment upon the carrying quality of the cherries. One

[1] Fisher, D. F. control of apple powdery mildew. U. S. Dept. Agr. Farmers' Bul. 1120, 14 p., 8 fig. 1920.

[2] The formula is given in sulphur, lime, arsenic sequence. 85 parts sulphur, no lime, and 15 parts arsenate of lead.

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or more 10-pound boxes of sound cherries from each plot were included in each shipping test, thus giving a minimum of approximately 1,000 cherries upon which to base any item of a count.

All the shipments were made by express, a part without ice and a part in pony refrigerators. The refrigerators held sixteen 10-pound boxes of fruit. They were well insulated and when properly iced maintained a temperature of 10° to 13° C. (50° to 55.4° F.), usually bringing the warm fruit down to this temperature in less than two days.

The results of the various shipping experiments are given in Tables I to VI and a summary in figure 2.

TABLE I.—*Effect of spraying Napoleon*[1] *and Black Republican*[2] *cherries, Salem, Oreg., 1915*

| Variety. | Orchard treatment. | Percentage of Monilia at picking time. | Condition of fruit after shipment and storage: Percentage of rot — Monilia. | Penicillium. | Rhizopus. | Percentage of sound fruit. |
|---|---|---|---|---|---|---|
| Napoleon | Bordeaux | 0.2 | 10.6 | 3.8 | 0 | 88.9 |
| | Self-boiled lime sulphur | .2 | 11.1 | 37.9 | 12.9 | 38.1 |
| | Untreated | .7 | 52.1 | 7.3 | 20.2 | 20.4 |
| Black Republican. | Bordeaux | .03 | 0.5 | 15.4 | 4.8 | 73.3 |
| | Self-boiled lime sulphur | .07 | 2.0 | 10.1 | 1.6 | 86.3 |
| | Commercial lime sulphur. | .05 | 7.8 | 12.1 | .03 | 80.1 |
| | Untreated | .03 | 17.3 | 3.7 | 1.3 | 77.7 |

[1] The Napoleon cherries were picked June 17, stored at 5° C. (41° F.) on June 18, removed and shipped by express without refrigeration June 27, received at Wenatchee, Wash., June 29, still practically free from rot, and held in a warm laboratory till July 2, when notes were taken.

[2] The Black Republican cherries were picked June 24, stored at 5° C. (41° F.) June 25, removed and shipped by express without refrigeration June 27, received at Wenatchee, Wash., June 29, still practically free from rot, and held in a warm laboratory till July 6, when notes were taken.

TABLE II.—*Effect of spraying Napoleon*[1] *and Black Republican*[2] *cherries, Salem, Oreg., 1916*

| Variety. | Orchard treatment. | Percentage of rot after shipment and storage: Monilia. | Penicillium. | Rhizopus. | Percentage of sound fruit. |
|---|---|---|---|---|---|
| Napoleon | Bordeaux | 12.6 | 7.4 | 0 | 80.0 |
| | Self-boiled lime-sulphur | 40.5 | 12.0 | 3.7 | 43.8 |
| | Commercial lime-sulphur | 21.5 | 16.2 | 2.4 | 59.9 |
| | Commercial lime-sulphur (last spraying omitted). | 64.5 | 4.1 | 20.0 | 11.4 |
| | Untreated | 80.8 | 16.7 | 0 | 2.5 |
| Black Republican. | Bordeaux | 19.6 | 4.0 | 1.1 | 75.3 |
| | Self-boiled lime-sulphur | 24.7 | .7 | .3 | 74.3 |
| | Commercial lime-sulphur | 14.2 | 14.2 | .6 | 71.0 |
| | Commercial lime-sulphur (last spraying omitted). | 36.9 | .8 | .2 | 62.1 |
| | Untreated | 35.0 | .1 | 0 | 64.9 |

[1] The Napoleon cherries were picked July 3, stored at 5° C. (41° F.) July 4, removed and shipped by express without refrigeration July 6, received at Wenatchee, Wash., July 8, and held in a warm laboratory till July 20, when notes were taken.

[2] The Black Republican cherries were picked July 6 to 10, stored at a temperature of 5° C. (41° F.) till July 14, shipped by express without refrigeration to Wenatchee, Wash., received July 16, and held in a warm laboratory till July 21, when notes were taken.

TABLE III.—*Effect of spraying Napoleon [1] and Lambert [2] cherries, 1916*

| Orchard treatment. | Percentage of rot after shipment and storage. | | | | | |
|---|---|---|---|---|---|---|
| | On Napoleon. | | | | On Lambert. | |
| | July 12. | | July 13. | | July 22. | |
| | Monilia. | Other rots. | Monilia. | Other rots. | Monilia. | Other rots. |
| Bordeaux | 2.2 | 0.2 | 6.0 | 0.8 | 5.6 | 4.2 |
| Self-boiled lime-sulphur | 11.0 | 0 | 23.8 | 1.2 | 5.7 | 1.6 |
| Commercial lime-sulphur | 4.0 | 0 | 7.5 | .6 | 7.0 | 11.6 |
| Commercial lime-sulphur (last spraying omitted) | 1.5 | 0 | 0.5 | 1.8 | 3.7 | 12.0 |
| Untreated | 14.8 | .1 | 37.7 | 1.6 | 21.0 | 3.6 |

[1] The Napoleon cherries were picked July 5 and shipped in pony refrigerators the same day, received in Washington, D. C., July 12, with ice pans empty and fruit warm and held without cooling till July 13.

[2] The Lambert cherries were picked July 14 and shipped in pony refrigerators the same day, received in Washington, D. C., July 21, and notes taken on July 22.

TABLE IV.—*Effect of spraying Napoleon,[1] Black Republican,[2] and Lambert [3] cherries, 1917*

| Orchard treatment. | Percentage of rot after shipment and storage. | | | | | | Percentage of rot after shipment on Lambert. | | |
|---|---|---|---|---|---|---|---|---|---|
| | On Napoleon. | | | On Black Republican. | | | | | |
| | Monilia. | Penicillium. | Rhizopus. | Monilia. | Penicillium. | Rhizopus. | Monilia. | Penicillium. | Rhizopus. |
| Bordeaux | 0 | 0.2 | 0 | 0.2 | 11.8 | 2.4 | 0.1 | 0.5 | 2.8 |
| Self-boiled lime-sulphur | .1 | 0 | 0 | 0 | 2.0 | 9.3 | 0 | 0 | 2.2 |
| As above, but last application omitted | .3 | .3 | 0 | 0 | .4 | 1.1 | .... | .... | .... |
| Commercial lime-sulphur | 0 | 0 | 1.3 | .1 | .3 | .1 | .6 | .3 | 4.0 |
| Untreated | .3 | .1 | .9 | 2.7 | .1 | 1.4 | 5.0 | .1 | 11.9 |

[1] The Napoleon cherries were picked July 7 and shipped in pony refrigerators the same day, received in Wenatchee, Wash., July 12, and held in a warm room till July 14, when notes were taken.

[2] The Black Republican cherries were picked July 15, packed in pony refrigerators the same day and shipped to Wenatchee, Wash., received July 18, held under ice till July 19 and then at room temperature till July 24, when notes were taken.

[3] The Lambert cherries were picked July 21, shipped in pony refrigerators the same day, received in Washington, D. C., July 30, when notes were taken.

TABLE V.—*Effect of spraying Napoleon cherries, 1918* [1]

| Lot | Orchard treatment. | Percentage of rot. | | | | | |
|---|---|---|---|---|---|---|---|
| | | On fruit picked and packed in the middle of the day. | | | On fruit picked and packed in the cool of the morning. | | |
| | | Monilia. | Penicillium. | Rhizopus. | Monilia. | Penicillium. | Rhizopus. |
| A.. | Bordeaux | 1.5 | 9.4 | 1.0 | ...... | ...... | ...... |
| | Lime-sulphur | 1.9 | 7.0 | 4.0 | ...... | ...... | ...... |
| | Lime-sulphur (last application omitted). | 1.9 | 6.0 | .3 | ...... | ...... | ...... |
| | Untreated | 4.3 | 9.3 | .6 | ...... | ...... | ...... |
| B.. | Bordeaux | .6 | 2.9 | 26.9 | 0 | 0.8 | 8.7 |
| | Bordeaux (last application omitted). | 0 | 1.6 | 39.0 | 0 | .2 | 52.3 |
| | Lime-sulphur | .2 | 2.7 | 4.6 | 0 | 1.6 | 67.4 |
| | Lime-sulphur (last application omitted). | .4 | 3.6 | 11.3 | 1.2 | .6 | 42.9 |
| | Untreated | 6.5 | 1.2 | 41.0 | 1.2 | .9 | 24.5 |
| C.. | Bordeaux | 0 | 12.0 | 15.1 | .2 | .9 | 21.4 |
| | Bordeaux (last application omitted). | .8 | 2.9 | 36.5 | 2.1 | .8 | 31.5 |
| | Lime-sulphur | 0 | .7 | 9.0 | .2 | 6.2 | 60.4 |
| | Lime-sulphur (last application omitted). | 1.5 | 5.0 | 22.0 | .4 | 3.2 | 19.0 |
| | Untreated | 10.0 | 1.9 | 33.7 | 2.8 | 5.8 | 64.8 |

[1] The cherries were picked June 26. Lot A was held 12 days at Salem, Oreg., in an open warehouse. Lot B was shipped in pony refrigerators to Wenatchee, Wash., held under ice till July 2, and without ice one day, notes being taken July 3. Lot C was shipped in pony refrigerators to Washington, D. C., arriving July 3, and held warm till July 5, when notes were taken.

TABLE VI.—*Effect of spraying Napoleon cherries, 1919* [1]

| Plot No. | Orchard treatment | Percentage of rot | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | July 3, after 6 days in transit | | July 15, after storage at 15° C. (59° F.) for 12 days. | | | July 15, after storage at 5° C. (41° F.) for 12 days. | | |
| | | Monilia. | Penicillium. | Monilia. | Penicillium. | Rhizopus. | Monilia. | Penicillium. | Rhizopus. |
| 1 | Bordeaux | 1.1 | 1.0 | 11.5 | 3.5 | 0 | 10.2 | 7.5 | 0 |
| 2 | Lime-sulphur | 1.0 | 1.2 | 13.8 | 4.5 | 0 | 12.3 | 4.2 | 0 |
| 3 | Sulphur dust | 14.7 | 22.8 | 9.3 | 37.0 | 35.2 | 19.4 | 26.5 | 0 |
| 4 | Untreated | 22.4 | 1.5 | 57.0 | 15.2 | 0 | 28.0 | .7 | 0 |

[1] The cherries were picked from plots 1 and 2 on June 25. A rain followed on June 26, and the cherries from plots 3 and 4 were picked on June 27. The rain probably resulted in there being relatively less protective material left on the dusted than on the sprayed fruit. The picked fruit from plots 1 and 2 was not placed under ice till June 27, and this delay in cooling may have partly or entirely offset any harmful effects from the rains received by plots 3 and 4. Two 10-pound boxes from each plot were shipped in a pony refrigerator to Washington, D. C., were received warm July 2, were held overnight at a temperature of approximately 7° C. (44.6° F.), and notes were taken July 3. The sound fruit was saved, and half of the cherries from each plot were stored at a constant temperature of 5° C. (41° F.) and half at a constant temperature of 15° C. (59° F.). On July 15 notes were taken on the amount of rot that had developed in storage.

A discussion of the results from the cherry experiments is given on pages 474–477.

## SPRAYED AND UNSPRAYED ITALIAN AND AGEN PRUNES IN TRANSIT AND IN STORAGE

The prune is best known as a dried product, but a considerable part of the western crop, especially from the irrigated districts, is shipped to the eastern markets as "green" or fresh prunes. The question of the development of rots is a very important one in such shipments, and it is also an important consideration when delays occur at the drying plants.

The spraying experiments were made in the orchards of A. W. Moody, Felida, Wash., and L. T. Reynolds, Salem, Oreg. Both Italian and Agen Petite or French) prunes were included in the tests. The spray materials were similar to those described for the cherries. In 1915, 1918, and 1919 a 4–4–50 Bordeaux was used, and in 1916 and 1917 a 2–4–50 Bordeaux. In 1916 to 1919, inclusive, a 50–35–15 sulphur dust [2] was used in the earlier applications and a 50–50–0 in the last. In 1919 a second sulphur dust plot was given an 85–0–15 mixture in the earlier application and an 85–15–0 mixture in the last, and two different brands of Bordeaux dust were tested.

In 1915 sprayings were made March 24, April 8, May 1, June 21, and August 6 in the first orchard, and May 29, June 21, and August 6 in the second orchard; in 1916, April 8 to 12, April 25 to 27, May 30 and August 30 at Felida, Wash., and April 1, April 21, and June 16 at Salem, Oreg., in 1917, April 28, May 18, June 15, and September 12; in 1918, April 11, April 29, May 27, and August 20; and in 1919, April 8, April 25, May 21, and August 23. The earlier applications (before May 10) were for the control of blossom infection and probably had little effect upon the occurrence of rot on the ripe fruit.

TABLE VII.—*Effect of spraying Italian prunes, Felida, Wash., 1915* [1]

| | Orchard treatment. | Percentage of Monilia rot at harvest. | Percentage of rot Sept. 21, after shipment and storage. | | | Percentage sound Sept. 21. |
|---|---|---|---|---|---|---|
| | | | Monilia. | Penicillium. | Rhizopus. | |
| First orchard | Bordeaux | 0 | 2.7 | 27.7 | 33.5 | 36.1 |
| | Self-boiled lime-sulphur. | 0.9 | 7.0 | 36.4 | 31.5 | 25.1 |
| | Commercial lime-sulphur. | .7 | 9.3 | 44.2 | 21.5 | 25.0 |
| | Untreated | 3.4 | 30.3 | 29.8 | 28.5 | 11.4 |
| Second orchard | Bordeaux | 4.2 | 35.7 | 22.7 | 28.8 | 12.8 |
| | Self-boiled lime-sulphur. | 3.3 | 27.8 | 25.4 | 38.9 | 7.9 |
| | Commercial lime-sulphur. | 4.8 | 20.6 | 32.6 | 17.5 | 29.3 |
| | Untreated | 5.4 | 56.0 | 14.1 | 28.6 | 1.3 |

[1] The trees in the first orchard were 24 years old, and those in the second 15. The prunes were harvested Sept. 7 to 10, shipped to Wenatchee, Wash., by ordinary express, and held without refrigeration till Sept. 21.

[2] Fifty pounds sulphur dust, 35 pounds of lime, and 15 pounds arsenate of lead.

Although brown rot usually caused heavy losses in the neighboring orchards, sometimes destroying more than 75 per cent of the crop, it was never serious even on the control plots in the orchards in which the experiments were made. Notes were taken on the amount of rot at picking time and also on the amount developed in shipment and in storage. The shipping tests were carried out as described for the cherries. The prunes used in the experiments were carefully picked from the tree several days before the drying season began. They were too green for drying, yet riper than the average "green" prune shipments.

TABLE VIII.—*Effect of spraying Italian prunes, Felida, Wash., 1916* [1]

| Orchard treatment. | Percentage of Monilia-rot at harvest. | Percentage of rot Sept. 19, after shipment | | | Percentage of rot Sept. 23, after shipment and warm storage | | |
|---|---|---|---|---|---|---|---|
| | | Monilia. | Penicillium. | Rhizopus. | Monilia. | Penicillium. | Rhizopus. |
| Bordeaux | 1.3 | 2.7 | 0.2 | 0.3 | 8.0 | 12.1 | 12.8 |
| Self-boiled lime-sulphur | 2.4 | .3 | .1 | .2 | 3.5 | 8.2 | 14.4 |
| As above, but last application omitted | 3.8 | 1.9 | .8 | 1.1 | 19.4 | 1.1 | 2.9 |
| Commercial lime-sulphur | 2.2 | 0 | 0 | 0 | 6.5 | 0 | 8.9 |
| Sulphur dust | 1.6 | .6 | .6 | 1.5 | 2.8 | 8.4 | 11.4 |
| Sulphur dust (last application omitted) | 4.7 | 2.7 | 0 | 2.8 | 5.4 | 7.1 | 11.0 |
| Untreated | 8.0 | 0 | 0 | 0 | 32.3 | 7.7 | 0 |

[1] The prunes were harvested Sept. 12 and Sept. 23. Fruit from the first picking was shipped in pony refrigerators to Washington, D. C., received in good condition Sept. 19, and held at room temperature till Sept. 23.

TABLE IX.—*Effect of spraying Italian prunes, Salem, Oreg., 1916* [1]

| Orchard treatment. | Percentage of rot after shipment and warm storage. | | |
|---|---|---|---|
| | Monilia. | Penicillium. | Rhizopus. |
| Bordeaux | 7.1 | 3.8 | 1.7 |
| Self-boiled lime-sulphur | 7.6 | 3.8 | 2.6 |
| Commercial lime-sulphur | 1.9 | 0 | .8 |
| Untreated | 12.9 | 0 | 0 |

[1] The prunes were practically free from rot in the orchard, were harvested Sept. 6, shipped by express without refrigeration to Wenatchee, Wash., and held at room temperature till Sept. 16, when notes were taken.

TABLE X.—*Effect of spraying Italian prunes, Felida, Wash., 1917*[1]

| Orchard treatment | Percentage of rot | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Monilia at picking time | | | After shipment to Wenatchee, Wash | | | After shipment to Washington, D C | |
| | First picking. | Second picking | Total | Monilia | Penicillium | Rhizopus | Monilia | Penicillium |
| Bordeaux | 3.1 | 0.4 | 1.6 | 2.3 | 15.6 | 0 | 10.5 | 82.9 |
| Self-boiled lime-sulphur | 1.5 | .3 | .6 | 1.5 | 10.8 | 0 | | |
| As above but last omitted | 4.0 | .7 | 1.5 | 1.8 | 48.1 | 0 | | |
| Lime-sulphur 1-50 | 4.6 | .4 | 2.0 | .3 | 31.7 | 0 | 12.7 | 84.8 |
| Sulphur dust | 6.9 | .8 | 3.8 | 2.8 | 36.2 | 0 | | |
| Untreated | 14.5 | 2.5 | 6.5 | 18.2 | 15.6 | 0 | 20.4 | 62.6 |

[1] The prunes were harvested Sept 22, to 26 and Oct 2 to 5 The shipment to Washington, D C, was made in a pony refrigerator, started Sept. 25, received Oct 11 with no ice and in such bad condition that several lots were discarded The shipment to Wenatchee, Wash, was by express without refrigeration, started Sept. 26, received Sept 28 and allowed to stand in a warm room till Sept. 30, when notes were taken.

TABLE XI.—*Effect of spraying Italian prunes, Felida, Wash., 1918*[1]

| Orchard treatment | Percentage of rot | | | | | | |
|---|---|---|---|---|---|---|---|
| | Monilia at picking time | After shipment to Wenatchee, Wash., and 8 days' storage at 15° C | | | After shipment to Washington, D C, under ice and 2 days' delay at 25° to 30° C. | | |
| | | Monilia. | Penicillium. | Rhizopus | Monilia. | Penicillium | Rhizopus |
| Bordeaux | 0.3 | 1.3 | 1.1 | 18.8 | 0 | 0.4 | 31.9 |
| Self-boiled lime-sulphur | .2 | 0 | 1.6 | 10.9 | 0 | 0 | 37.6 |
| As above but last omitted | .2 | .6 | 1.1 | 3.6 | 1.7 | 0 | 9.2 |
| Commercial lime-sulphur | .5 | 0 | .5 | 10.9 | 0 | .4 | 34.3 |
| As above but last omitted | .6 | .3 | .5 | .3 | 0 | .8 | 15.5 |
| Sulphur dust | .1 | .4 | 1.2 | 3.4 | 0 | 0 | 39.3 |
| Untreated | .3 | 1.5 | 1.2 | 10.1 | 0 | 1.5 | 24.3 |

[1] The prunes were harvested Sept. 6 and 11. The shipment to Washington, D C., was made in a pony refrigerator, started Sept. 6, received in good condition Sept. 14, and notes taken Sept. 16. The shipment to Wenatchee, Wash., was without refrigeration, started Sept. 11, received Sept. 12, and held in cellar storage till Sept. 20, when notes were taken.

TABLE XII.—*Effect of spraying Italian and Agen (Petite or French) prunes, Salem Oreg., 1919* [1]

| Variety | Orchard treatment | Percentage of rot. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Monilia at picking time. | After shipment to Wenatchee, Wash., without ice and 5 days' storage without ice, 18° C. (64.4° F.). | | | After shipment to Wenatchee, Wash., under ice and 5 days' storage under ice. | | | Total rot in the refrigerator shipment after the 5 days' cool storage had been followed by 2 days' warm storage. | | |
| | | | Monilia. | Penicillium. | Rhizopus. | Monilia. | Penicillium. | Rhizopus. | Monilia. | Penicillium. | Rhizopus. |
| Italian | Bordeaux | 1.6 | 29.9 | .... | 3.2 | 4.6 | 0 | 0 | 9.6 | 0 | 0 |
| | Self-boiled lime-sulphur | [2] .1 | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| | As above but last omitted | [2] 2.5 | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| | Commercial lime-sulphur | .6 | 12.5 | 2.1 | .9 | .7 | 0 | 0 | 3.9 | 0.4 | 0 |
| | Untreated | 8.3 | 71.8 | 1.3 | 1.3 | 17.5 | 0.5 | 0 | 36.1 | .6 | 0 |
| Agen | Self-boiled lime-sulphur | .1 | .... | .... | .... | .6 | 0 | 0 | 11.0 | 1.3 | 1.1 |
| | As above but last omitted | .2 | .... | .... | .... | 13.9 | 0 | 0 | 38.5 | 1.9 | 0 |
| | Sulphur dust 50-35-15 | .2 | .... | .... | .... | .5 | 0 | 0 | 10.4 | 1.2 | .2 |
| | Sulphur-dust 85-0-15 | .2 | .... | .... | .... | .2 | 0 | .02 | 7.6 | 1.1 | .8 |
| | Bordeaux dust A | .9 | .... | .... | .... | 2.8 | 0 | 1.3 | 20.2 | .8 | 1.6 |
| | Bordeaux dust B | 8.7 | .... | .... | .... | 1.4 | 0 | 0 | 34.6 | .2 | 0 |
| | Untreated | 1.8 | .... | .... | .... | 7.0 | 0 | 0 | 50.4 | .2 | 0 |

[1] The prunes were harvested Sept. 15, shipped to Wenatchee, Wash., Sept. 16, received Sept. 18, and notes taken Sept. 23.
[2] No shipment.

The contrast in the amount of brown rot (Monilia) on the fruit from the different plots after shipment and storage is shown graphically in figure 1.

A study of the figure shows that the unsprayed fruit developed six to nine times as much brown rot under transportation and market

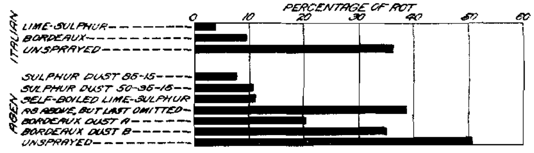


FIG. 1.—Brown rot on Italian and Agen prunes after shipment and storage. 1919.

conditions as the fruit from the plots receiving the best orchard treatment. The sulphur dust was as efficient as the sprays, but the Bordeaux dust was far less efficient.

The results show that orchard spraying may have great value on the market even when the amount of disease in the orchard has been negligible.

## DISCUSSION OF RESULTS

In order to obtain the composite results from the various prune and cherry experiments the data from the different spraying and shipping tests have been brought together and averaged. The results are shown in figures 2 to 6, inclusive.

Figure 2 shows the comparative efficacy of spraying and dusting as determined by the average of the four years' results on prunes. Little contrast is shown between the two methods of treatment, both sulphur dust and self-boiled lime-sulphur having reduced the amount of brown rot at picking time from 4 per cent to approximately 1 per cent and

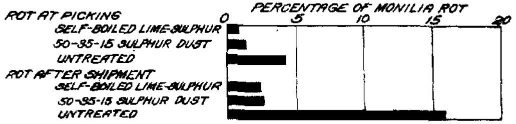


Fig. 2.—Comparative results from spraying and dusting in a four years' test on prunes

reduced the amount developed in shipments from 16 per cent to 2.5 per cent.

It was pointed out earlier in the paper that the different spray applications were probably not of equal value in the control of brown rot on the fruit. The great importance of the last application in this connection is shown graphically in figures 3 and 4. A reference to these figures

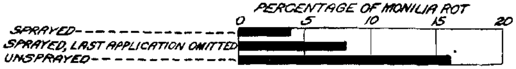


Fig. 3.—Brown-rot control of cherries as influenced by a late spray application (about three weeks before picking time). The average results obtained from 15 different shipping tests.

shows that with the prunes approximately one-half and with the cherries approximately one-third the brown-rot control was due to this late spraying.

The comparative results obtained with the different rots in the various shipping experiments are shown in figures 5 and 6. The term sprayed

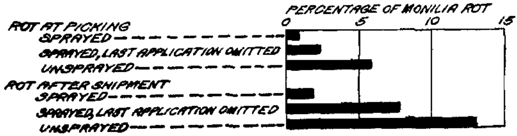


Fig. 4.—Brown rot control of prunes as influenced by a late application of spray or dust (three to five weeks before picking time). The average results obtained from 7 orchard experiments and 11 shipping tests.

as used in these two figures includes both dusting and spraying. As has already been pointed out, there was no year in which there was a serious epidemic of rot in the orchards under investigation. The amount of rot on the untreated cherries at picking time never ran as high as 1 per cent. The average amount of brown rot on the sprayed prunes at

picking time, as shown in figure 6, was 1.6 per cent and the average amount on the untreated prunes was 4.6 per cent. The orchard loss from rot with either the prunes or the cherries would be considered of very minor importance in practical operations, scarcely justifying the expense of spraying; yet even under these conditions the orchard spraying has shown decided beneficial effects in the carrying quality of the fruit in transportation and storage. The good effects, however, have been largely if not entirely confined to the control of Monilia rot. With both the prunes and cherries the unsprayed fruit has developed approximately four times as much of Monilia rot as the sprayed fruit, but has shown practically no greater susceptibility to Penicillium and Rhizopus rots. These contrasting results are in harmony with the nature of the different fungi. Monilia is a parasitic fungus and able to penetrate the sound skin of both ripe and green fruit; Penicillium and Rhizopus are saprophytic fungi, able to attack only the harvested fruit and dependent upon bruises and skin cracks for first points of entrance. The Monilia spores come primarily from the orchard, but Penicillium and Rhizopus have an almost universal distribution.

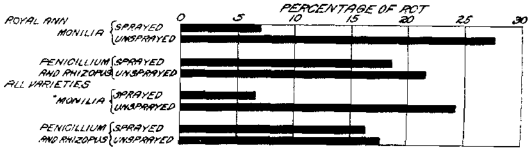


FIG. 5.—A comparison of the average development of rot on sprayed and unsprayed cherries in 18 different shipping and storage experiments.

Under such circumstances it would be expected that orchard spraying would furnish at least partial protection against brown rot in transportation and storage, since it would both decrease the supply of spores and furnish a more or less complete protecting film on the fruit. On the other hand, orchard spraying could not be expected to have any appreciable effect upon the spore supply of fungi like Penicillium and Rhizopus that are of general occurrence, and a film of spray on the skin could offer little protection against fungi that enter through breaks in the skin.

While these contrasts in the different fungi are of importance in connection with the present studies, it should not be inferred from the foregoing statements that skin punctures have no effect upon the occurrence of Monilia rot, for it is well known that any abuse to the fruit is decidedly favorable to the development of the disease;[1] nor should it be inferred that Rhizopus and Penicillium are entirely unable to penetrate the sound skin, for when these fungi are once well established in a crate they may spread out from a center of infection without much regard to the soundness of the adjacent fruit. This is particularly true of Rhizopus, and especially where it is favored by a high temperature. Under such a condition it often spreads through a package of stone fruit in a most rapid and indiscriminate manner.

[1] RAMSEY, H. J. THE HANDLING AND SHIPPING OF FRESH CHERRIES FROM THE WILLAMETTE VALLEY. U. S. Dept. Agr. Bul. 331, 28 p., 11 fig., 1916.

With all the rots temperature has been an extremely important factor. In the 1919 experiments (Table VI) part of the fruit was stored at 15° C. and part at 5°, with striking contrasts in the results. Rhizopus was entirely eliminated at the lower temperature, and Penicillium and Monilia were greatly reduced. Short shipments without refrigeration have resulted in heavy losses, while fresh fruit, both sprayed and unsprayed, has been shipped across the continent in pony refrigerators under ice with no decay upon arrival. Fruit that was free from rot after seven days in the refrigerators became badly decayed after standing one or two days in a warm room, the unsprayed fruit always developing the most rot but the sprayed fruit never remaining free from it. Refrigeration is always valuable; but it is evident that its importance increases with any decrease in orchard or packing-house care.

It is evident that there is a widely distributed responsibility for the occurrence of stone fruit rots in transit and in storage. Orchard spraying may be one of the important factors in the control of Monilia rot on the

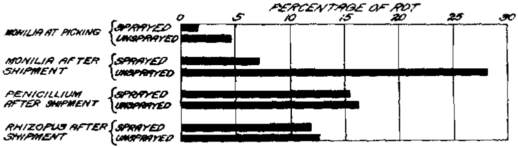


Fig. 6.—A comparison of the average development of rot on sprayed and unsprayed Italian prunes in 11 different shipping and storage experiments.

harvested fruit, but as a protection against Penicillium and Rhizopus rots it has little or no value.

## SUMMARY

(1) Orchard spraying has reduced the amount of Monilia or brown rot developed on sweet cherries in transportation and storage experiments from 24.3 to 6.4 per cent. All the cherries were from orchards where there was less than 1 per cent of rot on either the sprayed or unsprayed fruit at picking time.

(2) In similar shipping and storage experiments with Italian prunes there has been an average of 28 per cent of brown rot on the untreated fruit and 7.1 per cent on the sprayed or dusted fruit. The amount of rot on the unsprayed fruit at picking time was 4.6 per cent and on the sprayed fruit 1.6 per cent.

(3) About half the brown rot control secured in the shipping tests with prunes and about one-third of that secured with cherries was due to the spray application made three or four weeks before picking time.

(4) There has been little contrast between the brown rot control secured with sulphur dust and that secured with the standard spray materials.

(5) Spraying and dusting have had little or no effect upon the development of Penicillium and Rhizopus rots in transit and storage, their occurrence apparently being much more influenced by the prevalence of bruises and skin punctures.

(6) The unsprayed fruit has shown a greater need of refrigeration than the sprayed, and the injured fruit a greater need than the sound.

# STORAGE OF CONIFEROUS TREE SEED

By C. R. Tillotson

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During the period from 1909 to 1913 the United States Forest Service was especially active in its reforestation program. There were large areas of deforested land on the national forests, and there was a sincere desire on the part of the organization to serve the public interest by bringing these lands into a productive state as soon as possible. The program involved the growing in nurseries and planting of many millions of young trees each year and also the sowing of seed directly on extensive areas of deforested land. To carry out the program large quantities of seed were needed. In the year 1910 alone, 63,000 pounds of seed were collected. Foresters know that seed is not borne in the same abundance upon trees each year. A good seed crop in any region is often followed by one to several very poor or lean crops. It accordingly is desirable to collect during years of plenty seed in large enough quantities to last several years. The Forest Service did pursue this course and thereupon became confronted with the problem of how to store the seed so that it would not deteriorate greatly in germinative ability and energy before it could be used. This problem was not a new one. European foresters had been faced with it a good many years ago and had made substantial progress in its solution for some species. One of the most intensive sets of European experiments with coniferous seed was perhaps that of Dr. Adolf Cieslar [1] which was begun in the spring of 1886 and continued over a period of 11 years. Dr. Cieslar attempted to determine not only the effect of air-tight storage on seeds of Norway spruce, black (Austrian) and white pine, but also the effect of heating these seeds before placing them in storage. He came to the conclusions that (1) storing under air-tight covering lengthens the life of these species of seed so that when stored in this way they often show a considerably higher germination percentage, especially in the later years of storing, than seeds of the same origin stored in the air, this difference in favor of air-tight storing of seed amounting to 33 per cent in the case of 6-year-old Norway spruce seed; (2) storing seed away from the air also results in higher germinating power; (3) the application of heat at 45° to 55° C. to seeds of white and black pine at the beginning of storing injures the viability of these seeds and also their germinating power to a considerable extent, but Norway spruce is injured less by this means, and its germinating power is even kept at a high point by strong heating; (4) heating at 30° to 40° C. for one hour has a not unfavorable effect on the seed of these three species. When stored away from the air, such seed maintains both viability and germinating energy at as high a point as that of unheated seed; in fact, the slightly heated seed shows in later years of storing a tendency to germinate in a very rapidly rising curve.

[1] Cieslar, Adolf. versuche über aufbewahrung von nadelholzsamen unter luftdichtem verschlusse. *In* Centlbl. Gesam. Forstw., Bd. 23, Heft 4, p. 167-174. 1897.

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Experiments conducted by the German Chief Forester Haack [1] at the Eberswald Forest Academy from 1906 to 1909 confirm some of the results of Dr. Cieslar and give additional information on the subject of seed storage. His experiments were confined to seed of Scotch pine. He found that exclusion of air can not wholly prevent a loss of germinating power with increasing age of the seed. This is manifested less by a final decrease of germinating percentage than by a falling off in germinating energy. But compared with that of seed stored in the air, this decrease of germinating power is extremely slight. After three years the air-tight seed had a germinating power of nearly 90 per cent as against 22 to 70 per cent for seed stored in the open air in the same room.

Another conclusion of Haack's was that in no case should seed that has been shut up in air-tight containers without previous thorough drying be placed in a storeroom in which the temperature is likely to increase, even if only occasionally (for instance, in attics). This conclusion was based on two experiments. In one, two air-tight bottles were placed on thermostats heated to 36° C. and left for eight weeks. One of the bottles contained air-dried seed, the other seed from which 5 per cent of its weight in moisture had been removed in an exsiccator. In the test, the former germinated only a little over 1 per cent, the latter 96 per cent. In a similar experiment at a temperature of 30° C., the germination test at the end of four months gave 40 per cent and 92 per cent, respectively. It might have been just as well for Chief Forester Haack to emphasize the necessity of thorough but not excessive drying of the seed before placing them in air-tight containers rather than stress the point of storing in a fairly cool room not subject to a rise in temperature. Such rooms are to be had only by some special arrangement. Later in the same article, Haack does state that in storing pine seed air-tight, care should be taken that the seed is neither moist nor overdry. He decided that the degree of dryness which the seed has when it comes from the kiln or when spread out in a well-heated room or dried in the sun would probably be best—about 1 to 2 per cent lighter than its average weight in the ordinary seed bin. His experiments showed that long-continued drying is harmful to pine seed. The germinating power of Scotch pine seed left in the exsiccator for four years fell to 16 per cent at the end of that time, while the same seed not dried in this way still showed a germination of 80 per cent.

Haack conducted one experiment to determine the effect of different temperatures of the storeroom upon seed in air-tight containers. Scotch pine was stored for three years in a room heated to a temperature of 20° to 25° C., in an unheated room, and in a cellar 1 meter in depth Both cellar and unheated room were free from frost in winter; in summer the unheated room was somewhat warmer than the cellar. The results indicated that a temperature of 20° to 25° C. in the heated room was injurious to the seed. The germination percentage dropped from 96 to 82 in the three years. With strong seed there was very little decrease in germination of seed stored either in the basement or in the unheated room. With weak seed, however, not thoroughly dried before being placed in the container, there was, after three years, a difference in germination of 20 per cent in favor of the seed stored in the basement.

These experiments of Dr. Cieslar and Chief Forester Haack, while thorough in themselves, were confined to only four species, three of

[1] HAACK (Otto H. A.). DER KIEFERSAMEN. VERHÄLTNIS ZWISCHEN KEIMPROZENT UND PRAKTISCHEM WERT. MEHRJÄHRIGE AUFBEWAHRUNG OHNE VERMINDERUNG DES KEIMPROZENTS. *In* Ztschr. Forst. u. Jagdw., Jahrg. 41, Heft 6, p. 353-381, 1 fig. 1909.

them European. In order to meet an immediate need for information, it seemed desirable to extend and expand upon them somewhat to those American species most used in reforestation operations on the national forests. These species were western yellow pine (*Pinus ponderosa* Law.), western white pine (*Pinus monticola* Dougl.), white pine (*Pinus strobus* Linn.), Engelmann spruce (*Picea engelmanni* Engelm.), Douglas fir (*Pseudotsuga taxifolia* (Law.) Britton), and lodgepole pine (*Pinus contorta* Loud). The study should now be followed up with those more sensitive coniferous seeds, the true firs, the cedars, arborvitas, redwoods, and the numerous species of American hardwoods of which so little is known.

The study brings a realization of the fact that it is a mistake to deal with so many variables in an intensive project of this nature. It is difficult if not impossible to be sure of the cause or causes for any particular result. There is now a need for further investigations of slightly smaller scope, in which the variables will be reduced to a minimum and in which by laboratory methods, the exact physiological, chemical, and any other changes which the seeds undergo can be followed closely. These changes almost surely will throw light upon the behavior of seed in storage.

## FACTORS AFFECTING EXPERIMENTS

Some of the conditions under which this study was carried out should be stated. Because of a shifting in personnel the study in various stages has come under the direction of several men. This has not been conducive to the best development of the project, and it may be that the analyses of the results are not so thorough as though made by the one who conceived the study. Apparently through oversight, no tests of the seed were made before they were put in storage. It is not known, accordingly, to what extent deterioration progressed in the seed during its first year of storage. That there was deterioration in the case of most containers is shown by the very general superiority after one year of the seed stored in air-tight bottles. Because of this lack of an original test, it has, in making analyses of the results, been necessary to use as the basis of comparison, the germination of seed after storage for one year in the air-tight bottles.

On account of the large number of variable factors involved (6 species of seed, 5 kinds of containers, 13 storage points, and 3 temperature conditions at each of these points), the general conclusions are by no means fully supported by the results in every individual test. It is thought, however, that the average results are a safe criterion of what may in general be expected of these coniferous seed in storage.

Fresh seed, with the wings removed, of the species previously mentioned was obtained during the fall and winter of 1908–9 in the amounts and from the sources indicated below:

*Picea engelmanni*, 10 pounds, San Isabel National Forest. Colorado.
*Pinus monticola*, 55 pounds, Coeur d'Alene National Forest, Idaho.
*Pinus contorta*, 12 pounds, Deerlodge National Forest. Montana.
*Pinus ponderosa*, 70 pounds, Boise National Forest, Idaho.
*Pinus strobus*, 30 pounds, New York State.
*Pseudotsuga taxifolia*, 25 pounds, San Isabel National Forest, Colorado.

When the seed was all brought together at Washington, D. C., it was spread out thinly on a floor and fanned steadily for two days by means of an electric fan. The object was to dry the seed coats thoroughly.

Each lot of seed was then divided roughly into portions of about 600 to 800 seeds each, and these were distributed equally among the following containers:

1. Ordinary manila paper coin envelopes.
2. Similar envelopes soaked in melted paraffin.
3. Cotton cloth bags.
4. Similar bags soaked in boiled linseed oil and dried.
5. Glass bottles which after filling were sealed air-tight with paraffin.

Seed of all six species stored in each of the five containers constituted one test set of samples. For convenience in handling, shipping, and storing, each test set was placed in a small wooden box lined with a wire mesh to prevent the access of rodents.

## POINTS OF STORAGE

It was one purpose of this study to determine whether seed deteriorated in storage to a greater extent in one geographical region than in another.

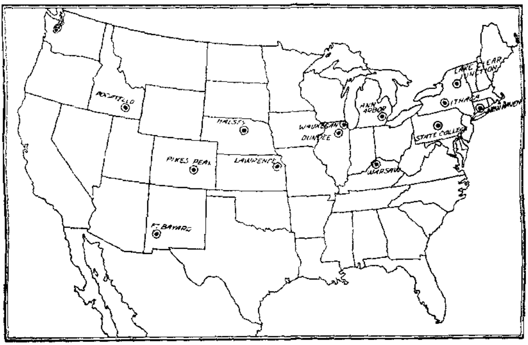


FIG. 1.—Map showing points at which coniferous seeds were stored to test effect of geographical location

Thirteen points of storage, accordingly, as indicated below and on the map (fig. 1), were selected. These, it will be noted, are rather widely scattered over the United States and afford a fair basis for arriving at some conclusions concerning this particular aspect of seed storage.

| POINT OF STORAGE | APPROXIMATE ALTITUDE |
|---|---|
| 1. Ann Arbor, Mich | 875 feet |
| 2. Dundee, Ill | 700 feet |
| 3. Fort Bayard, N. Mex | 6,500 feet |
| 4. Halsey, Nebr | 2,700 feet |
| 5. Ithaca, N. Y | 800 feet |
| 6. Lake Clear Junction, N. Y | 1,600 feet |
| 7. Lawrence, Kans | 800 feet |
| 8. New Haven, Conn | 30 feet |
| 9. Pikes Peak, Colo | 9,000 feet |
| 10. Pocatello, Idaho | 4,500 feet |
| 11. State College, Pa | 1,150 feet |
| 12. Warsaw, Ky | 400 feet |
| 13. Waukegan, Ill | 600 feet |

## CONDITIONS OF STORAGE

Another point on which it was hoped this study would throw some light was the effect of several conditions of temperature on seed in storage. At each of the geographical points mentioned, accordingly, the cooperators in the study were requested to store the seed where each of the following conditions of temperature would prevail:

1. Ordinary indoor temperature, such as an office shelf where the temperature would always be above the freezing point.
2. Fluctuating temperature, as in an outbuilding or unheated garret where the temperature would follow rather closely the actual outdoor variations. Proximity to a stable was to be avoided.
3. Fairly uniform low temperature, such as prevails in an unheated basement or cellar.

To just what extent these conditions obtained and were entirely comparable at all points of storage, it is not possible to say. It would not be surprising if there were considerable differences, particularly in the low-temperature conditions. It is believed, however, that they were similar enough to warrant the drawing of general conclusions from the tests.

## PERIOD COVERED BY STUDY

The study was planned to cover a period of approximately five years. The seed was sent to the 13 points of storage during March, 1909. In January, 1910, and again in January, 1911, 1912, and 1914, three test sets (one stored at each of the three temperature conditions) were forwarded by express from each of the storage points to Washington, D. C., for testing.

It is thus seen that tests were carried on after the seed had been in storage for periods of approximately one, two, three, and five years. There was no test of seed in storage for four years. From a practical standpoint, at least, it seemed that tests covering a period of five years would be sufficient. It is unlikely that seed in commercial quantities at least will be stored for a longer time. As a matter of interest, however, a few of the seeds which had been stored in bottles were carried over for another five years and tested during the year 1919. This phase of the study will be taken up in more detail later (p. 510).

## SEED-TESTING OPERATION

The seed-testing operation was a simple but rather large undertaking. There were carried on during each of the four years 195 tests for each of the six species. Two hundred seeds were used in each test. Ordinary greenhouse wooden flats about 14 by 18 by 4 inches in depth were nearly filled with fresh sand, which was compacted and smoothed off; the seed for each test was scattered uniformly over the surface, pressed into the sand by means of a board, and then covered with ⅛ inch to ¼ inch of sand. The flats were then set on greenhouse benches where the sand was kept moist during the course of germination by sprinkling it with an ordinary watering pot equipped with a fine rose or spraying nozzle. The seeds were protected from mice by covering the flats with frames made of fly screen. Ants were troublesome at first, but they were successfully combatted by scattering napthalene flakes on the benches. During the winter and spring months when artificial heat was employed in the greenhouse, the temperature sought was about 70° F

during the daytime and about 50° at night. There were, of course, some variations in this, particularly as summer approached. On bright, sunshiny days in late spring or early summer, the temperature in the greenhouse sometimes approached 100° during the middle of the day. By that time, however, the germination tests were practically completed for all except the slow-germinating eastern and western white pines. A careful day by day record was kept of the germination. As the seeds sprouted and developed a short radicle they were plucked out of the sand and discarded. While this method of conducting germination tests for all kinds of seed, particularly those which germinate very slowly like the white pines, is not considered ideal, the results secured for the several years are at least comparative.

## CONCLUSIONS

In noting the conclusions, the reader should keep in mind that they have reference to coniferous seed only, and that they are based upon the results of one series of tests with only six species of coniferous seed and may not accordingly be applicable to all coniferous seed, or even to the same kinds of seed from other sources. It should also be remembered that the seed used in this experiment was thoroughly air-dried before it was placed in air-tight storage.

(1) Storage of coniferous seed in the air-tight bottle is far superior in every respect to storage in any other container. The average germination for the 5-year period of seed stored in bottles over that stored in the next best container was 22 per cent.

(2) Thoroughly air-dried coniferous seed stored in air-tight bottles is little if at all affected by such differences in temperatures as exist between a location where the temperature follows the natural fluctuations, a location indoors where the temperature never falls below freezing, and a location in an ordinary cellar or basement.

(3) Coniferous seed stored in air-tight bottles is little if at all affected by the geographic location of the storage point.

(4) The quality of coniferous seed, by which is meant its value in terms of both germinative energy and germinative ability, is much superior in the case of seed stored in an air-tight bottle to that stored in any other receptacle. This is manifest even at the end of one year of storage.

(5) Following the air-tight bottle, the various containers, in the order of their merit, fall into the following sequence: paper bag paraffined, paper bag, cloth bag, and oiled cloth bag. It should be noted that an ordinary paper bag closed at the top is superior to a cloth bag for seed storage. The oiled cloth bag is practically worthless as a container.

(6) The use of any of the containers except the air-tight bottle results in such rapid deterioration after one or two years of storage under the temperature conditions of this experiment as to render the seed, particularly of Engelmann spruce, Douglas fir, and white pine, of very little worth.

(7) Storage at the indoor temperature is superior to that at the fluctuating or low. Storage at the low temperature shows the poorest results. This low temperature has reference not to a low uniform temperature of freezing or less but to that of an ordinary cellar or basement. The difference in germination percentage is not great under these three conditions but is sufficient to make indoor storage preferable to the other two conditions.

(8) Some geographic locations are more favorable for seed storage than others. Fort Bayard, Pikes Peak, Pocatello, and Lake Clear Junction—all points of relatively high altitudes and, with the possible exception of Lake Clear Junction, of low relative humidities—stand out as exceptionally favorable localities. Four middle-western points, Waukegan, Dundee, Lawrence, and Warsaw, and one Atlantic seaboard point, New Haven, stand out as unfavorable localities for seed storage. Such points should apparently be avoided where ordinary methods of storage are followed. No one of the geographic locations shows marked superiority over another when the seeds are stored in air-tight bottles.

(9). In respect to sustained vitality, the seeds employed in this study range themselves in the following sequence, with the strongest first: western yellow pine, lodgepole pine, western white pine, white pine, Engelmann spruce, and Douglas fir.

## WHAT THE STUDY SHOWS

The points brought out by the study can be shown better, it is thought, by the accompanying tables and curves with a few comments than by lengthy discourse.

### EFFECT OF CONTAINER

Table I, together with the curves (fig. 2), brings out what was very evident during the progress of the study, the striking superiority of the seeds stored in the air-tight bottles over those stored in any other container. This is particularly true when the storage period extends beyond one year and is more striking in the case of Engelmann spruce, Douglas fir, and white pine than in that of lodgepole, western yellow, and western white pines. The seeds of the former three species are apparently more likely to deteriorate than those of the latter three and after two years of storage are of little worth.

It seems safe to assume (barring any hypothesis of post-ripening of the seed during storage) that the germination of the seed before it was put in storage was at least equal to that of the seed stored in bottles at the end of one year. Based on this assumption, Table I shows that the average deterioration for all species has at the end of five years been for seed stored in a paper bag 45 per cent; in a paper bag paraffined, 42.3 per cent; in a cloth bag, 47.8 per cent; in a cloth bag oiled, 51.4 per cent; and in the air-tight bottle, 10.8 per cent. In this connection it should be noted that *Pinus ponderosa* stored four years, *Picea engelmanni* and *Pseudotsuga taxifolia* three years, and *Pinus contorta* two years show little if any decrease in the total germination of bottle-stored seed. In fact, germination at the end of two and three years has in some cases been greater than at the end of one year. The behavior of *Pinus strobus* and *Pinus monticola* seed is a puzzle. It will be noted that the germination percentage of bottle-stored seed decreases through the second and third years, but at the end of the fifth year (1914) it equals or betters that of the second year (1911). The 1914 germination of these two species with seed stored in the other four containers is practically equal to the 1912 germination. Possibly the conditions for germination in 1914 were somewhat superior to those in 1912, or there may have been some physiological development to account for it.

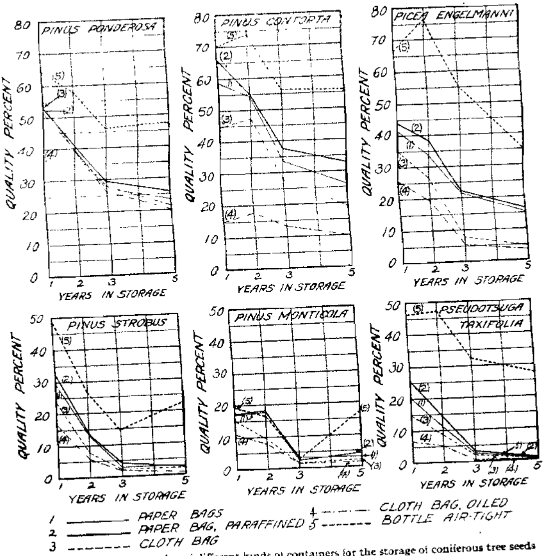


FIG. 2.—Graphs showing value of different kinds of containers for the storage of coniferous tree seeds

TABLE I.—*Germination percentages of seed stored in different containers and under different temperature conditions*

| Temperature and container. | Seed tested for— 30 days. | 33 days. | 33 days. | 34 days. | | Seed tested for— 95 days. | 80 days. | 93 days. | 96 days. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Picea engelmanni.* | | | | | *Pinus contorta* | | | | |
| | 1910 | 1911 | 1912 | 1914 | Average for all 4 years | 1910 | 1911 | 1912 | 1914 | Average for all 4 years. |
| Fluctuating temperature. | | | | | | | | | | |
| Paper bag | 47.0 | 38.8 | 20.3 | 10.2 | 29.2 | 66.0 | 55.1 | 40.8 | 24.6 | 49.1 |
| Paper bag, paraffined | 40.4 | 43.2 | 25.0 | 11.1 | 29.9 | 74.0 | 62.3 | 45.2 | 44.0 | 56.4 |
| Cloth bag | 37.7 | 27.5 | 15.0 | 6.7 | 21.7 | 55.0 | 47.3 | 32.2 | 26.9 | 40.4 |
| Cloth bag, oiled | 26.7 | 20.9 | 11.6 | 8.3 | 16.9 | 18.2 | 13.5 | 15.0 | 13.5 | 15.0 |
| *Bottle, air-tight* | 67.1 | 79.7 | 70.0 | 54.4 | 67.8 | 75.0 | 73.2 | 60.0 | 59.8 | 67.0 |
| Average for all containers | 43.8 | 42.0 | 28.4 | 18.2 | | 57.8 | 50.1 | 38.6 | 35.8 | |
| Average for all containers for all 4 years | | | | | 33.1 | | | | | 45.6 |
| Indoor temperature. | | | | | | | | | | |
| Paper bag | 52.1 | 45.7 | 31.1 | 7.4 | 34.1 | 68.8 | 61.2 | 44.6 | 36.9 | 52.9 |
| Paper bag, paraffined | 55.1 | 51.5 | 30.1 | 9.2 | 36.5 | 77.4 | 57.4 | 49.9 | 45.9 | 57.6 |
| *Cloth bag* | 53.3 | 39.5 | 15.3 | 7.9 | 29.0 | 42.2 | 51.2 | 34.6 | 21.2 | 37.3 |
| Cloth bag, oiled | 38.7 | 31.8 | 15.3 | 4.5 | 22.6 | 16.6 | 20.8 | 16.2 | 9.5 | 15.8 |
| Bottle, air-tight | 67.8 | 74.7 | 73.6 | 61.9 | 69.5 | 72.1 | 75.8 | 58.4 | 54.9 | 65.3 |
| Average for all containers | 53.0 | 48.6 | 33.5 | 18.2 | | 55.4 | 53.4 | 40.7 | 33.7 | |
| Average for all containers for all 4 years | | | | | 38.3 | | | | | 45.8 |
| Low temperature | | | | | | | | | | |
| Paper bag | 30.2 | 19.2 | 10.5 | 2.7 | 15.7 | 63.9 | 40.6 | 30.7 | 29.0 | 43.3 |
| *Paper bag, paraffined* | 40.8 | 23.0 | 11.3 | 4.7 | 20.1 | 70.7 | 40.6 | 34.0 | 37.0 | 47.8 |
| Cloth bag | 24.3 | 15.9 | 6.3 | 1.8 | 12.1 | 49.2 | 40.4 | 31.6 | 29.1 | 39.1 |
| Cloth bag, oiled | 16.1 | 8.3 | [illegible] | 1.2 | 6.5 | 12.7 | 16.6 | 14.5 | 13.5 | 14.3 |
| Bottle, air-tight | 67.5 | 77.2 | 70.0 | 57.8 | 69.6 | 72.9 | 77.2 | 64.2 | 61.8 | 69.0 |
| Average for all containers | 35.8 | 28.8 | 20.9 | 13.6 | | 53.9 | 47.9 | 35.0 | 34.1 | |
| Average for all containers for all 4 years | | | | | 24.8 | | | | | 42.7 |
| *Average for all 3 temperatures:* | | | | | | | | | | |
| Paper bag | 43.1 | 34.6 | 20.6 | 6.8 | 26.3 | 66.2 | 55.3 | 38.7 | 33.5 | 48.4 |
| Paper bag, paraffined | 45.4 | 39.4 | 22.1 | 8.3 | 28.8 | 74.0 | 56.4 | 43.0 | 42.3 | 53.9 |
| Cloth bag | 37.8 | 27.6 | 12.9 | 5.5 | 21.0 | 48.8 | 48.3 | 32.8 | 25.7 | 38.9 |
| Cloth bag, oiled | 27.2 | 20.3 | 9.1 | 4.7 | 15.3 | 15.6 | 17.0 | 15.2 | 12.2 | 15.5 |
| Bottle, air-tight | 67.5 | 77.2 | 73.2 | 58.0 | 69.0 | 73.3 | 75.4 | 60.9 | 58.8 | 66.9 |
| Average for all containers and temperatures | 44.2 | 39.8 | 27.6 | 16.7 | | 55.6 | 50.5 | 38.1 | 34.5 | |

ABLE I.—*Germination percentages of seed stored in different containers and under different temperature conditions*—Continued

| Temperature and container | *Pseudotsuga taxifolia* 1910 | 1911 | 1912 | 1914 | Average for all 4 years. | *Pinus ponderosa* 1910 | 1911 | 1912 | 1914 | Average for all 4 years. |
|---|---|---|---|---|---|---|---|---|---|---|
| Seed tested for— | 30 days. | 34 days. | 34 days | 34 days. | | 105 days | 117 days | 117 days. | 113 days. | |
| Fluctuating temperature | | | | | | | | | | |
| Paper bag.. | 22.7 | 14.7 | 6.9 | 0.5 | 11.2 | 76.0 | 63.9 | 55.5 | 45.3 | 60.2 |
| Paper bag, paraffined | 25.7 | 20.3 | 6.6 | 1.4 | 13.5 | 75.6 | 61.1 | 58.1 | 50.2 | 61.4 |
| Cloth bag | 14.2 | 11.5 | 6.3 | .1 | 8.0 | 75.2 | 63.7 | 54.8 | 42.4 | 59.0 |
| Cloth bag, oiled | 3.9 | 6.6 | 1.8 | .1 | 3.1 | 71.6 | 60.6 | 43.7 | 32.6 | 53.4 |
| Bottle, air-tight | 44.3 | 51.5 | 45.5 | 30.4 | 42.9 | 78.3 | 72.2 | 74.2 | 72.8 | 74.4 |
| Average for all containers | 24.2 | 20.9 | 13.4 | 6.5 | .. | 75.3 | 64.3 | 55.3 | 48.7 | ... |
| Average for all containers for all 4 years | .. | | | | 15.8 | ... | . | .. | | 61.6 |
| Indoor temperature. | | | | | | | | | | |
| Paper bag. | 28.7 | 14.5 | 4.3 | .4 | 12.0 | 75.2 | 67.7 | 62.6 | 51.5 | 64.3 |
| Paper bag, paraffined | 34.8 | 17.7 | 6.0 | .9 | 14.8 | 77.1 | 67.8 | 63.6 | 58.1 | 66.7 |
| Cloth bag | 24.7 | 12.3 | 3.7 | .5 | 10.3 | 77.2 | 65.8 | 62.6 | 44.6 | 62.6 |
| Cloth bag, oiled | 11.3 | 3.3 | .9 | .7 | 4.1 | 74.4 | 61.8 | 56.4 | 38.3 | 57.7 |
| Bottle, air-tight | 49.1 | 48.6 | 45.8 | 38.6 | 43.0 | 76.1 | 72.1 | 75.3 | 76.6 | 75.0 |
| Average for all containers.. | 29.7 | 19.3 | 12.1 | 6.2 | .. | 76.0 | 67.0 | 64.1 | 53.8 | . |
| Average for all containers for all 4 years | .. | .. | . | | 16.8 | ..... | .. | .. | ... | 65.2 |
| Low temperature. | | | | | | | | | | |
| Paper bag | 13.5 | 7.7 | .7 | 1.3 | 5.8 | 72.1 | 48.1 | 42.6 | 32.5 | 48.8 |
| Paper bag, paraffined | 19.6 | 9.8 | 2.0 | 1.4 | 8.4 | 71.8 | 53.4 | 50.5 | 32.6 | 52.1 |
| Cloth bag | 8.5 | 9.0 | .3 | 1.6 | 4.1 | 73.9 | 49.2 | 38.9 | 29.9 | 48.0 |
| Cloth bag, oiled. | 6.5 | 6.0 | ...... | .1 | 3.4 | 68.3 | 50.5 | 39.4 | 37.6 | 49.0 |
| Bottle, air-tight | 49.8 | 50.8 | 47.7 | 33.9 | 45.6 | 78.5 | 67.3 | 75.9 | 75.0 | 74.2 |
| Average for all containers. | 19.6 | 16.2 | 10.3 | 7.7 | .. | 72.9 | 53.7 | 49.5 | 41.5 | .. |
| Average for all containers for all 4 years.. | ..... | .. | . | . | 13.5 | ... | ...... | ... | ... | 54.4 |
| Average for all 3 temperatures: | | | | | | | | | | |
| Paper bag. | 21.6 | 12.3 | 4.0 | .7 | 9.7 | 74.4 | 59.9 | 53.6 | 43.1 | 57.8 |
| Paper bag, paraffined | 26.6 | 15.9 | 5.1 | 1.2 | 12.2 | 74.8 | 60.8 | 57.4 | 47.0 | 60.0 |
| Cloth bag | 15.8 | 9.9 | 3.4 | .7 | 7.5 | 75.4 | 59.6 | 52.1 | 39.0 | 56.5 |
| Cloth bag, oiled | 7.2 | 5.6 | .0 | .3 | 3.5 | 71.4 | 57.6 | 48.2 | 36.2 | 47.9 |
| Bottle, air-tight. | 47.7 | 50.3 | 46.3 | 31.0 | 43.8 | 77.6 | 70.5 | 75.2 | 74.8 | 74.5 |
| Average for all containers and temperatures.... | 23.8 | 18.8 | 11.9 | 6.8 | ..... | 74.7 | 61.7 | 57.3 | 48.0 | ....... |

ABLE I.—*Germination percentages of seed stored in different containers and under different temperature conditions*—Continued

| Temperature and container. | Seed tested for— | | | | | Seed tested for— | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 115 days. | 126 days. | 129 days. | 126 days. | | 115 days. | 120 days. | 129 days. | 126 days. | |
| | *Pinus strobus.* | | | | | *Pinus monticola* | | | | |
| | 1910 | 1911 | 1912 | 1914 | Average for all 4 years. | 1910 | 1911 | 1912 | 1914 | Average for all 4 years. |
| Fluctuating temperature: | | | | | | | | | | |
| Paper bag | 40.6 | 27.5 | 8.8 | 9.8 | 21.7 | 36.0 | 24.7 | 0.2 | 6.0 | 18.1 |
| Paper bag, paraffined | 44.0 | 29.9 | 9.9 | 8.9 | 23.2 | 36.7 | 27.0 | 5.7 | 7.5 | 19.2 |
| Cloth bag | 30.5 | 18.4 | 8.3 | 8.6 | 16.5 | 30.3 | 11.3 | 3.0 | 4.4 | [illegible] |
| Cloth bag, oiled | 24.5 | 7.9 | 5.0 | 2.2 | 9.9 | 18.7 | 10.4 | 4.7 | 3.0 | 9.2 |
| Bottle, air-tight | 56.0 | 46.5 | 30.7 | 49.2 | 45.6 | 41.1 | 27.7 | 7.8 | 25.9 | 25.6 |
| Average for all containers | 39.1 | 26.0 | 12.5 | 15.7 | .... | 32.6 | 20.2 | 5.5 | 9.4 | |
| Average for all containers for all 4 years | | | | | 23.3 | | | | | 16.9 |
| Indoor temperature: | | | | | | | | | | |
| Paper bag | 40.6 | 28.7 | 9.6 | 4.4 | 22.3 | 36.2 | 26.3 | 7.0 | 6.2 | 18.9 |
| Paper bag, paraffined | 49.9 | 32.9 | 12.8 | 4.0 | 24.9 | 39.2 | 24.2 | 7.3 | 9.9 | 20.2 |
| Cloth bag | 35.0 | 13.4 | 5.8 | 3.2 | 14.4 | 32.1 | 15.1 | 3.8 | 4.3 | 13.8 |
| Cloth bag, oiled | 23.2 | 11.2 | 5.1 | 1.7 | 10.3 | 22.0 | 13.0 | 3.6 | 3.6 | 10.6 |
| Bottle, air-tight | 58.5 | 50.6 | 33.6 | 50.7 | 48.4 | 39.7 | 24.5 | 9.2 | 33.9 | 20.8 |
| Average for all containers | 42.6 | 27.4 | 13.4 | 12.8 | ..... | 33.8 | 20.6 | 6.2 | 11.6 | ... |
| Average for all containers for all 4 years | | | | | 24.1 | | | | | 18.1 |
| Low temperature: | | | | | | | | | | |
| Paper bag | 31.7 | 23.3 | 4.9 | 5.0 | 16.2 | 33.6 | 17.9 | 6.0 | 3.3 | 15.2 |
| Paper bag, paraffined | 37.8 | 27.9 | 9.0 | 4.6 | 19.8 | 34.7 | 22.2 | 6.2 | 5.4 | 17.1 |
| Cloth bag | 27.3 | 12.2 | 1.2 | 2.2 | 10.7 | 26.7 | 10.7 | 3.7 | 2.2 | 10.8 |
| Cloth bag, oiled | 16.2 | 6.8 | .7 | .8 | 6.1 | 19.3 | 6.2 | .9 | 1.3 | 6.9 |
| Bottle, air-tight | 58.9 | 48.9 | 27.0 | 46.2 | 45.3 | 44.8 | 22.8 | 7.7 | 28.8 | 26.0 |
| Average for all containers | 34.4 | 23.8 | 8.6 | 11.8 | ..... | 31.8 | 16.0 | 4.9 | 8.2 | . |
| Average for all containers for all 4 years | | | | | 19.6 | | | | | 15.2 |
| Average for all 3 temperatures: | | | | | | | | | | |
| Paper bag | 39.6 | 26.5 | 7.8 | 6.4 | 20.1 | 35.3 | 23.0 | 6.4 | 5.2 | 17.5 |
| Paper bag, paraffined | 43.9 | 30.2 | 10.6 | 5.8 | 22.6 | 36.9 | 24.5 | 6.4 | 7.6 | 18.9 |
| Cloth bag | 30.9 | 14.7 | 5.1 | 4.7 | 13.9 | 29.7 | 12.4 | 3.5 | 3.6 | 12.5 |
| Cloth bag, oiled | 21.3 | 8.6 | 3.6 | 1.6 | 8.8 | 20.0 | 9.9 | 3.1 | 2.6 | 8.9 |
| Bottle, air-tight | 57.8 | 48.7 | 30.4 | 48.7 | 46.4 | 41.9 | 25.0 | 8.2 | 29.5 | 26.2 |
| Average for all containers and temperatures | 38.7 | 25.7 | 11.5 | 13.4 | ...... | 32.7 | 18.9 | 5.5 | 9.7 | ...... |

TABLE I.—*Germination percentages of seed stored in different containers and under different temperature conditions*—Continued

| Temperature and containers. | Average for all species. | | | | Average for all 4 years for all species. |
|---|---|---|---|---|---|
| | 1910 | 1911 | 1912 | 1914 | |
| Fluctuating temperature. | | | | | |
| Paper bag | 48.1 | 37.5 | 23.1 | 17.8 | 31.6 |
| Paper bag, paraffined | 49.4 | 40.6 | 25.1 | 20.5 | 33.9 |
| Cloth bag | 40.5 | 30.0 | 19.9 | 14.9 | 26.3 |
| Cloth bag, oiled | 27.3 | 20.0 | 14.5 | 10.0 | 18.0 |
| Bottle, air-tight | 60.3 | 58.5 | 51.1 | 48.8 | 54.6 |
| Average for all containers | 45.1 | 37.3 | 26.7 | 22.4 | 32.9 |
| Indoor temperature | | | | | |
| Paper bag | 51.3 | 40.7 | 26.5 | 17.8 | 34.1 |
| Paper bag, paraffined | 55.5 | 41.9 | 28.3 | 21.3 | 36.8 |
| Cloth bag | 43.7 | 32.9 | 21.3 | 13.6 | 27.9 |
| Cloth bag, oiled | 31.1 | 23.7 | 16.3 | 9.7 | 20.2 |
| Bottle, air-tight | 60.6 | 57.7 | 49.3 | 51.1 | 54.7 |
| Average for all containers | 48.4 | 39.4 | 28.3 | 22.7 | 34.7 |
| Low temperature | | | | | |
| Paper bag | 40.8 | 27.6 | 15.9 | 12.3 | 24.2 |
| Paper bag, paraffined | 45.9 | 31.1 | 18.9 | 14.3 | 27.6 |
| Cloth bag | 35.0 | 23.4 | 13.7 | 11.1 | 20.8 |
| Cloth bag, oiled | 28.2 | 15.0 | 9.3 | 9.1 | 14.4 |
| Bottle, air-tight | 62.1 | 57.4 | 49.8 | 50.6 | 55.0 |
| Average for all containers | 41.4 | 31.1 | 21.5 | 19.5 | 28.4 |
| Average for all 3 temperatures | | | | | |
| Paper bag | 46.7 | 35.3 | 21.8 | 16.0 | 30.0 |
| Paper bag, paraffined | 50.3 | 37.9 | 24.1 | 18.7 | 32.8 |
| Cloth bag | 39.7 | 28.8 | 18.3 | 13.2 | 25.0 |
| Cloth bag, oiled | 27.3 | 20.0 | 13.4 | 9.6 | 17.6 |
| Bottle, air-tight | 61.0 | 57.7 | 50.1 | 50.2 | 54.8 |

Possibly the germination test was not continued long enough to determine the comparative germinative ability of the seed. When the test of bottle-stored seed was continued in 1912 for 270 days, an average germination of 58 per cent for *Pinus monticola* and 76 per cent for *Pinus strobus* resulted. This is in contrast to germination percentages of 8.2 and 30.4 for the 129-day period shown by Table I. Again, in 1914 when the test was continued for 155 days, the average germination percentages were 42 and 58.5, as contrasted with 26.2 and 48.7 for the 126-day period, also shown in Table I. When the germination figures are curved, however, they indicate that had the tests been continued longer during the years 1910 and 1911, there would have been a considerable increase in the germination percentage shown by the tests of those years. It is believed, accordingly, that the figures in the table are a very fair criterion of the comparative value or condition of the seed for the years in question. This does not explain the reason for the sudden jump in germination of bottle-stored seed in 1914. Frankly, the writer is not able to assign any definite reason. The delayed germination in nurseries of these two seeds, particularly *Pinus monticola*, always is a source of trouble, and it is not surprising that in these tests they have not followed a course similar to the other seed. There is much to be learned about these two species.

In addition to pointing out the very evident superiority of air-tight storage, Table I shows that the other containers should in respect to their merit be placed in the following sequence: paper bag paraffined, paper bag,

cloth bag, cloth bag oiled. This is somewhat interesting. Small lots of seed of a few pounds are perhaps stored more often in a cloth bag than in any other container. This study indicates that on the average an ordinary heavy manila type of paper bag would, if tied at the top, be superior to the cloth bag. If the paper bag can be treated with a coat of paraffin, it will be still better. In fact the germination percentage of *Pinus contorta* in the paraffined paper bag was slightly greater than, and of *P. ponderosa* nearly equal to, that of the sealed bottle after one year's storage. The oiled cloth bag is decidedly inferior. This is much less pronounced with *P. ponderosa* than with the other species. Whether the general inferiority of this container is due to penetration of the seed coat by the oil and consequent injury of the embryo, to the prevention of access of water to the embryo when the seed was sown, or to some other cause is unknown. Regardless of the reason, the truth is evident that such a container should be avoided in storing any of these seeds.

The superiority of the bottled seeds over those in other containers is in all probability due to the almost complete suspension of physiological activity by the seed thus stored. (This was not verified by any experiments undertaken in connection with this study.) The two conditions essential for such activity are warmth and moisture. The seed in the bottles was of course exposed to high enough temperature to induce respiration, but the necessary amount of moisture for any great degree of activity was not present. Such moisture as was present in the seed or bottled air could not be increased by additions from without. On the other hand, the seed in the other containers was intermittently subjected to both temperature and atmospheric moisture conditions sufficient at times to induce rather active respiration. Such respiration can be carried on only by using up food material stored in the seed itself, with the consequent gradual weakening of its germinative ability. The seed stored in the paraffined paper bag and the plain paper bag were, it is believed, less subject to changes of atmospheric moisture than that stored in the ordinary cloth bag. The rate of deterioration was in consequence less rapid.

It is quite generally held by forest tree seed investigators that the true criterion of the quality of seed is not alone its germinative ability or viability (germination percentage) but rather this germinative ability in conjunction with germinative power or energy. Rapidity of germination (the germination percentage at the end of a certain period of time) is the measure of this germinative energy. This period is measured from the date of sowing the seed through the time that germination is proceeding steadily and rapidly and at the end of which it starts to fall off rather abruptly. If the progress of germination is plotted and curved, the point at which the curve begins to fall off rather abruptly and flatten out will represent the number of days which should be selected for that species. Now, the quality of the seed will be determined by the mean of the germination at the end of this period and the final germination percentage.

By final germinative percentage is not meant here the absolute final but rather that at the end of a reasonable period. Accepting this hypothesis, Table II was prepared, and the curves in figure 1 were drawn from it. They represent the quality of each species of seed stored in each container at the end of one, two, three, and five years. The periods

selected for the measure of germinative energy and final germination were as follows:

| | Period of germinative energy | Period of final germination |
|---|---|---|
| Engelmann spruce | 15 | 30 |
| Douglas fir | 15 | 30 |
| Lodgepole pine | 55 | 80 |
| Western yellow pine | 45 | 105 |
| White pine | 35 | 115 |
| Western white pine | 45 | 115 |

The germinative energy periods were selected through the use of the germination curves as the guide. Other investigators may not agree with these periods, and the writer will admit that other lots of seeds might indicate periods of different length. The period of final germination is the same as that shown in Table I for the year 1910. It was necessary for comparative results to select a period not longer than that over which the tests were conducted in any one of the four years. The tests were discontinued sooner in 1910 than in any other year, and this led to the selection of the test period during that year.

TABLE II.—*Quality of seed after storage for 1, 2, 3, and 5 years*

| Species | Container | Quality expressed in percentage after storage for— | | | |
|---|---|---|---|---|---|
| | | *1 year.* | *2 years.* | *3 years.* | *5 years.* |
| *Picea engelmanni* | Paper bag | 42.0 | 33.8 | 11.7 | 5.4 |
| | Paper bag paraffined | 44.2 | 38.6 | 12.4 | 6.6 |
| | Cloth bag | 35.6 | 26.8 | 7.8 | 4.4 |
| | Cloth bag oiled | 26.0 | 19.3 | 5.5 | 3.6 |
| | Bottle air-tight | 66.6 | 76.6 | 55.3 | 35.0 |
| *Pinus ponderosa* | Paper bag | 52.2 | 30.8 | 27.3 | 23.4 |
| | Paper bag paraffined | 53.8 | 41.0 | 29.6 | 25.8 |
| | Cloth bag | 54.9 | 39.6 | 28.0 | 21.5 |
| | Cloth bag oiled | 36.4 | 39.7 | 26.3 | 20.1 |
| | Bottle air-tight | 64.8 | 58.0 | 46.1 | 47.5 |
| *Pinus contorta* | Paper bag | 50.0 | 54.3 | 33.6 | 26.1 |
| | Paper bag paraffined | 66.4 | 55.2 | 37.6 | 32.8 |
| | Cloth bag | 44.7 | 47.4 | 27.7 | 19.2 |
| | Cloth bag oiled | 15.0 | 17.8 | 13.3 | 9.9 |
| | Bottle air-tight | 70.4 | 73.9 | 56.3 | 55.8 |
| *Pseudotsuga taxifolia* | Paper bag | 20.8 | 11.2 | 2.8 | .5 |
| | Paper bag paraffined | 26.0 | 14.6 | 3.3 | .9 |
| | Cloth bag | 15.0 | 8.8 | 2.2 | .5 |
| | Cloth bag oiled | 6.8 | 5.2 | .6 | .2 |
| | *Bottle air-tight* | 46.3 | 47.2 | 32.4 | 27.6 |
| *Pinus strobus* | Paper bag | 29.9 | 13.2 | 3.0 | 3.0 |
| | Paper bag paraffined | 32.9 | 13.6 | 4.2 | 2.5 |
| | Cloth bag | 24.0 | 7.8 | 2.2 | 2.2 |
| | Cloth bag oiled | 17.0 | 5.8 | 2.0 | .8 |
| | Bottle air-tight | 49.6 | 26.6 | 14.8 | 22.6 |
| *Pinus monticola* | Paper bag | 17.0 | 17.8 | 3.2 | 3.1 |
| | Paper bag paraffined | 17.3 | 18.2 | 2.4 | 4.4 |
| | Cloth bag | 15.4 | 9.4 | 1.6 | 2.2 |
| | Cloth bag oiled | 11.0 | 7.8 | 1.4 | 1.5 |
| | Bottle air-tight | 19.3 | 16.6 | 2.9 | 17.2 |

These curves are interesting. They emphasize more than ever the superiority of air-tight storage over any of the other methods. It will be recalled that, judged by final germination percentage only (Table I), the quality of lodgepole and western yellow pine seed stored in paraffined paper bags was at the end of one year practically equal to that of seed stored in the bottles. The curves adequately dispel this idea of equality. The bottle-stored seed of western yellow pine and lodgepole pine excels that in paraffined paper bags by 11 and 4 per cent, respectively. Furthermore, at the end of five years the bottle-stored seed of all species except western yellow pine is practically equal or superior to that stored for only one year in cloth bags, and the bottle-stored seed of western yellow pine is superior to that stored for two years in any of the other containers. Douglas fir, Engelmann spruce, and lodgepole pine seed stored in bottles, western yellow pine in oiled cloth bags, lodgepole pine in cloth and oiled cloth bags, and western white pine in paper and paraffined paper bags show some appreciation in quality at the end of the second year over that at the end of the first; there is in general a marked and fairly uniform deterioration of seed for a 3-year period, after which it is less rapid; the previously expressed relative merits of the various containers is confirmed—that is, in the order of their merit they should be ranged in the sequence of air-tight bottle, paper bag paraffined, paper bag, cloth bag, and oiled cloth bag. The oiled cloth bag is so inferior that it should receive no consideration at all for seed storage purposes.

## EFFECT OF TEMPERATURE

Of the three conditions of temperature under which the seed was stored, Table I clearly indicates that the highest average germination percentages were secured with that stored at the indoor temperature, followed in order by the fluctuating and low temperatures. The differences in the average germination percentage for the indoor and fluctuating temperatures is only 1.8 per cent, but the superiority of indoor temperature conditions over those in an ordinary basement or cellar is indicated by an average excess germination of 6 per cent. This general superiority, it will be noted from the table, is consistent with all of the species involved. Here again, however, the superiority of air-tight storage is evidenced by the fact that the seed stored in bottles at the low temperature shows no inferiority but in fact a slight superiority (0.3 and 0.4 per cent) over that stored under indoor and fluctuating temperature conditions.

Leaving the general averages in Table I and analyzing the results in Tables IV to IX, it will be noted that the superiority of seed stored at the indoor over that stored at the fluctuating and low temperatures seems to vary with the species about as follows: Engelmann spruce, Douglas fir, western yellow pine, western white pine, eastern white pine, and lodgepole pine. It will be noted that the two species, Engelmann spruce and Douglas fir, most susceptible to deterioration in storage were the most favorably affected by storage at the indoor temperature. The better results from storing at the indoor temperature was most marked at Halsey, New Haven, Lawrence, and Ann Arbor. Poor germination resulting from storage at low temperature was most pronounced at New Haven, Pikes Peak, Pocatello, Waukegan, Halsey, and State College.

TABLE III.—*Germination percentages of seed at the several storage points*

| Location. | *Picea engelmanni.* | | | | | *Pseudotsuga taxifolia.* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1910 | 1911 | 1912 | 1914 | Average | 1910 | 1911 | 1912 | 1914 | Average. |
| Ann Arbor | 53.7 | 44.9 | 26.5 | 13.2 | 35.0 | 20.2 | 16.7 | 10.2 | 8.5 | 13.9 |
| Dundee | 25.7 | 18.5 | 15.9 | 12.9 | 18.2 | 13.1 | 13.8 | 9.9 | 9.4 | 11.6 |
| Fort Bayard | 65.7 | 66.5 | 50.8 | 30.5 | 54.9 | 33.2 | 34.4 | 18.8 | 13.3 | 24.9 |
| Halsey | 45.6 | 44.7 | 22.5 | 11.4 | 31.1 | 24.6 | 15.9 | 8.9 | 4.3 | 13.4 |
| Ithaca | 43.5 | 43.9 | 24.9 | 14.4 | 31.2 | 28.1 | 17.3 | 11.7 | 3.7 | 15.2 |
| Lake Clear Junction | 51.3 | 56.7 | 35.9 | 16.3 | 40.1 | 37.6 | 31.1 | 15.1 | 6.0 | 22.4 |
| Lawrence | 22.1 | 18.4 | 12.4 | 3.4 | 14.1 | 13.6 | 11.9 | 9.7 | 2.6 | 9.4 |
| New Haven | 42.7 | 29.3 | 19.1 | 6.1 | 24.3 | 18.5 | 11.0 | 9.3 | 5.9 | 11.2 |
| Pikes Peak | 59.6 | 53.8 | 54.2 | 43.0 | 52.7 | 30.4 | 28.0 | 21.6 | 9.3 | 22.3 |
| Pocatello | 49.5 | 61.6 | 47.5 | 25.0 | 45.9 | 33.0 | 25.0 | 14.7 | 6.7 | 20.0 |
| State College | 46.4 | 42.5 | 19.6 | 14.1 | 26.2 | 22.8 | 12.5 | 9.2 | 4.8 | 12.4 |
| Warsaw | 20.0 | 15.6 | 11.6 | 12.3 | 17.4 | 13.7 | 11.0 | 6.3 | 6.0 | 9.8 |
| Waukegan | 40.5 | 33.4 | 20.9 | 14.0 | 27.2 | 20.4 | 15.5 | 10.4 | 6.1 | 13.1 |

| Location | *Pinus contorta.* | | | | | *Pinus ponderosa.* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1910 | 1911 | 1912 | 1914 | Average | 1910 | 1911 | 1912 | 1914 | Average |
| Ann Arbor | 50.0 | 36.8 | 44.2 | 22.9 | 40.0 | 77.6 | 59.3 | 68.5 | 61.5 | 66.7 |
| Dundee | 54.9 | 51.2 | 36.9 | 28.2 | 42.8 | 75.2 | 69.9 | 52.8 | 22.6 | 55.1 |
| Fort Bayard | 64.2 | 60.7 | 46.5 | 51.9 | 55.6 | 78.1 | 75.1 | 69.8 | 72.7 | 73.9 |
| Halsey | 50.9 | 57.1 | 39.9 | 32.4 | 45.1 | 73.8 | 72.5 | 61.6 | 60.6 | 67.1 |
| Ithaca | 53.5 | 53.6 | 39.7 | 30.2 | 44.2 | 75.5 | 67.4 | 64.8 | 63.5 | 67.8 |
| Lake Clear Junction | 54.9 | 50.5 | 40.5 | 40.6 | 46.1 | 75.8 | 66.9 | 70.1 | 65.2 | 69.5 |
| Lawrence | 58.5 | 43.8 | 30.8 | 22.5 | 38.9 | 68.8 | 58.4 | 44.8 | 24.2 | 49.0 |
| New Haven | 56.1 | 44.6 | 38.3 | 29.0 | 42.1 | 74.4 | 45.8 | 38.7 | 28.2 | 46.8 |
| Pikes Peak | 66.1 | 58.1 | 50.2 | 55.6 | 57.5 | 75.7 | 49.2 | 56.6 | 57.4 | 59.7 |
| Pocatello | 63.8 | 54.4 | 41.8 | 44.1 | 51.0 | 75.8 | 61.1 | 64.6 | 64.9 | 66.6 |
| State College | 48.7 | 50.2 | 42.1 | 32.5 | 40.9 | 71.8 | 52.7 | 66.5 | 49.1 | 60.0 |
| Warsaw | 50.3 | 39.9 | 24.3 | 24.3 | 34.7 | 76.1 | 52.3 | 35.2 | 19.9 | 45.8 |
| Waukegan | 48.5 | 55.8 | 34.3 | 33.3 | 42.7 | 76.1 | 69.8 | 68.5 | 47.3 | 65.4 |

| Location. | *Pinus strobus* | | | | | *Pinus monticola.* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1910 | 1911 | 1912 | 1914 | Average | 1910 | 1911 | 1912 | 1914 | Average. |
| Ann Arbor | 36.4 | 20.3 | 10.5 | 10.2 | 20.9 | 32.8 | 11.0 | 6.4 | 11.9 | 15.5 |
| Dundee | 29.3 | 21.8 | 8.2 | 10.9 | 17.6 | 30.8 | 19.9 | 5.6 | 9.3 | 16.4 |
| Fort Bayard | 51.8 | 44.9 | 29.3 | 16.7 | 35.7 | 42.0 | 52.1 | 6.5 | 27.9 | 32.3 |
| Halsey | 40.3 | 24.9 | 10.1 | 10.1 | 21.4 | 33.9 | 37.9 | 6.1 | 5.9 | 21.0 |
| Ithaca | 39.1 | 26.9 | 8.7 | 10.2 | 21.2 | 31.0 | 28.7 | 5.1 | 7.0 | 18.0 |
| Lake Clear Junction | 48.9 | 36.0 | 13.5 | 14.[illegible] | 28.2 | 31.1 | 19.8 | 5.8 | 12.7 | 17.4 |
| Lawrence | 20.7 | 10.0 | 7.9 | 11.0 | 12.4 | 23.1 | 6.5 | 4.6 | 7.3 | 10.9 |
| New Haven | 35.2 | 15.7 | 6.8 | 7.9 | 16.4 | 35.3 | 11.6 | 1.6 | 5.4 | 13.5 |
| Pikes Peak | 49.9 | 35.0 | 19.9 | 30.6 | 33.9 | 38.2 | 10.1 | 7.1 | 10.0 | 17.9 |
| Pocatello | 54.3 | 40.5 | 19.0 | 20.0 | 33.5 | 35.9 | 14.0 | 10.3 | 9.1 | 17.3 |
| State College | 36.9 | 23.5 | 9.4 | 9.4 | 19.8 | 33.9 | 14.0 | 6.9 | 4.3 | 14.8 |
| Warsaw | 23.4 | 9.9 | 5.6 | 11.4 | 12.6 | 27.6 | 5.3 | 2.1 | 4.7 | 10.0 |
| Waukegan | 35.0 | 19.5 | 7.6 | 9.8 | 18.1 | 29.3 | 13.7 | 4.6 | 4.3 | 12.9 |

| Location. | Average of all species. | | | | Average of all species for all 4 years. |
|---|---|---|---|---|---|
| | 1910 | 1911 | 1912 | 1914 | |
| Ann Arbor | 40.1 | 32.5 | 28.0 | 21.4 | 32.0 |
| Dundee | 38.2 | 32.5 | 21.6 | 15.6 | 27.0 |
| Fort Bayard | 55.8 | 55.6 | 38.0 | 35.5 | 46.2 |
| Halsey | 44.8 | 42.2 | 24.8 | 20.8 | 33.2 |
| Ithaca | 45.1 | 39.2 | 25.8 | 21.5 | 32.9 |
| Lake Clear Junction | 49.6 | 43.5 | 30.2 | 25.8 | 37.3 |
| Lawrence | 34.5 | 25.2 | 18.4 | 11.8 | 22.5 |
| New Haven | 43.7 | 26.3 | 19.0 | 13.8 | 25.7 |
| Pikes Peak | 53.3 | 39.0 | 34.9 | 35.3 | 40.6 |
| Pocatello | 52.0 | 42.9 | 33.0 | 28.3 | 39.0 |
| State College | 43.4 | 30.9 | 24.0 | 19.0 | 29.3 |
| Warsaw | 36.8 | 22.3 | 34.5 | 13.1 | 21.7 |
| Waukegan | 41.7 | 34.6 | 24.2 | 19.1 | 29.9 |

TABLE IV.—*Germination percentages of seed of Picea engelmanni at various storage points*

[Leaders indicate "no germination"]

| Storage point and temperature condition. | 1910, 28-day test. | | | | | | 1911, 33-day test. | | | | | | 1912, 33-day test. | | | | | | 1914, 14-day test. | | | | | | Average for all containers for all 4 years. | Average for each container at all 3 temperatures for all 4 years | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. |
| Ann Arbor, Mich.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 37.5 | 48.5 | 39.5 | 39.5 | 60.5 | 45.1 | 53.0 | 42.0 | 30.0 | 15.0 | 85.0 | 45.0 | 21.5 | 20.0 | 2.0 | 1.0 | 79.0 | 24.7 | ... | ... | ... | ... | 45.0 | 9.0 | 31.0 | | | | | |
| Indoor | 63.5 | 68.0 | 76.0 | 57.0 | 66.0 | 66.1 | 48.5 | 52.0 | 41.5 | 44.0 | 58.0 | 48.8 | 41.0 | 46.5 | 2.5 | 2.0 | 63.0 | 31.0 | 1.0 | 2.5 | ... | ... | 77.0 | 16.1 | 40.5 | | | | | |
| Low | 52.5 | 63.0 | 48.0 | 22.5 | 64.0 | 50.0 | 51.5 | 51.0 | 19.5 | 7.5 | 75.0 | 40.9 | 30.5 | 29.5 | 9.5 | ... | 76.0 | 29.1 | ... | ... | ... | ... | 72.0 | 14.4 | 33.0 | | | | | |
| Average | 51.2 | 59.8 | 54.5 | 39.7 | 63.5 | 53.7 | 51.0 | 48.3 | 30.3 | 22.2 | 72.7 | 44.9 | 31.0 | 32.0 | 4.7 | 1.0 | 72.7 | 28.3 | .3 | .8 | ... | ... | 64.7 | 13.2 | 35.0 | 33.4 | 35.2 | 22.4 | 15.7 | 68.4 |
| Dundee, Ill.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 18.0 | 26.0 | ... | .5 | 67.5 | 22.4 | 3.0 | 13.0 | 2.5 | ... | 78.0 | 19.3 | ... | 1.0 | ... | ... | 80.5 | 16.3 | ... | ... | | | 70.5 | 14.1 | 18.0 | | | | | |
| Indoor | 18.5 | 37.0 | 18.5 | ... | 67.5 | 28.3 | 1.5 | 4.0 | ... | ... | 87.5 | 18.6 | | 2.0 | | | 81.0 | 16.6 | ... | ... | ... | ... | 71.5 | 14.3 | 19.0 | | | | | |
| Low | 19.5 | 32.0 | 3.0 | ... | 77.5 | 26.4 | 1.5 | 2.5 | | ... | 84.0 | 17.6 | | | | | 74.0 | 14.8 | ... | | | | 52.0 | 10.4 | 17.3 | | | | | |
| Average | 18.6 | 31.7 | 7.2 | .2 | 70.8 | 25.7 | 2.0 | 6.5 | .8 | ... | 83.2 | 18.5 | | 1.0 | | . | 78.5 | 15.9 | ... | ... | | | 64.7 | 12.9 | 18.2 | 5.2 | 9.8 | 2.0 | .05 | 74.3 |
| Fort Bayard, N. Mex.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 54.0 | 59.5 | (a) | 64.5 | 77.0 | 63.8 | 67.0 | 82.5 | 61.0 | 15.0 | 79.0 | 60.9 | 34.0 | 59.0 | 18.0 | 17.0 | 81.0 | 45.8 | 11.0 | 8.0 | 9.0 | 9.0 | 40.0 | 15.4 | 43.3 | | | | | |
| Indoor | 63.5 | 68.5 | 67.5 | 71.0 | 69.0 | 67.9 | 71.5 | 74.5 | 75.0 | 60.5 | 77.0 | 73.3 | 64.0 | 71.5 | 64.0 | 63.0 | 77.0 | 67.9 | 33.0 | 32.5 | 46.0 | 21.5 | 68.0 | 40.2 | 62.4 | | | | | |
| Low | 61.5 | 63.0 | 69.0 | 63.0 | 69.0 | 65.1 | 70.0 | 60.0 | 67.0 | 54.5 | 74.0 | 65.1 | (b) | (b) | (b) | (b) | (b) | (b) | 23.5 | 45.5 | 20.5 | 14.0 | 75.5 | 35.8 | [c]55.3 | | | | | |
| Average | 59.7 | 63.7 | 68.3 | 66.2 | 71.7 | 65.7 | 69.5 | 72.3 | 67.7 | 46.3 | 76.7 | 66.5 | 49.0 | 65.2 | 51.0 | 40.0 | 79.0 | 56.8 | 22.5 | 28.7 | 23.2 | 14.8 | 61.2 | 30.5 | 54.9 | 50.4 | 57.5 | 33.0 | 41.8 | 71.2 |
| Halsey, Nebr.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 68.5 | 41.0 | 46.5 | 14.0 | 61.0 | 46.2 | 44.0 | 45.5 | 33.5 | 24.0 | 83.5 | 46.1 | 34.5 | 15.5 | 12.0 | 1.5 | 68.5 | 26.4 | ... | ... | | | 32.5 | 6.5 | 31.3 | | | | | |
| Indoor | 64.5 | 61.0 | 56.5 | 45.0 | 70.5 | 59.5 | 69.0 | 70.0 | 63.0 | 52.5 | 81.0 | 67.1 | 31.5 | . | 1.0 | 1.5 | 78.0 | 22.4 | 4.0 | .5 | . | ... | 65.5 | 14.0 | 40.8 | | | | | |
| Low | 25.0 | 50.0 | 6.0 | 1.5 | 73.0 | 31.1 | 2.5 | 10.0 | .5 | 1.0 | 82.0 | 21.0 | 4.5 | 8.0 | 1.0 | . | 79.5 | 18.6 | .5 | .5 | . | | 67.5 | 13.7 | 21.1 | | | | | |
| Average | 52.7 | 50.7 | 36.3 | 20.2 | 68.2 | 45.6 | 38.5 | 44.8 | 32.3 | 25.8 | 82.2 | 44.7 | 23.5 | 7.8 | 4.7 | 1.0 | 75.3 | 22.5 | 1.5 | .2 | . | | 55.2 | 11.4 | 31.1 | 29.0 | 25.9 | 18.3 | 11.8 | 70.2 |

[a] Seed rotten. [b] Not sown. [c] Average for 3 years.

TABLE IV.—*Germination percentages of seed of Picea engelmanni at various storage points*—Continued

[Leaders indicate "no germination"]

| Storage point and temperature condition. | 1910, 26-day test. | | | | | | 1911, 33-day test. | | | | | | 1912, 33-day test. | | | | | | 1914, 34-day test. | | | | | | Average for all containers for all 4 years. | Average for each container at all temperatures for all 4 years. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. |
| **Ithaca, N. Y.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 62.5 | 62.0 | 51.5 | 35.0 | 66.0 | 55.4 | 37.0 | 49.0 | 21.5 | 30.0 | 70.5 | 42.6 | 20.5 | 23.5 | 1.0 | | 68.0 | 22.6 | ... | ... | | | 72.5 | 14.5 | 33.5 | | | | | |
| Indoor | 38.0 | 39.0 | 52.5 | 42.5 | 63.0 | 47.0 | 59.5 | 65.5 | 52.0 | 52.0 | 66.0 | 59.0 | 50.5 | 51.5 | 8.0 | 1.5 | 71.0 | 36.5 | ... | 1.0 | ... | ... | 70.0 | 14.2 | 39.2 | | | | | |
| Low | 18.5 | 32.0 | 26.5 | 1.5 | 61.5 | 28.0 | 12.0 | 29.5 | 4.0 | .5 | 79.0 | 25.0 | .5 | .5 | ... | | 76.5 | 35.5 | (a) | (a) | (a) | (a) | (a) | (a) | [b] 22.8 | | | | | |
| **Average** | 39.7 | 44.3 | 43.5 | 26.3 | 63.5 | 43.5 | 36.2 | 48.0 | 25.8 | 27.5 | 71.8 | 41.9 | 23.8 | 25.2 | 3.0 | .5 | 71.8 | 24.9 | ... | .5 | ... | ... | 71.3 | 14.4 | 31.2 | 24.9 | 29.5 | 18.5 | 13.6 | 69.6 |
| **Lake Clear Junction, N. Y.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 60.0 | 26.0 | 66.5 | 7.5 | 74.5 | 46.9 | 69.0 | 71.0 | 63.5 | 37.0 | 82.0 | 64.5 | 17.5 | 40.0 | 4.0 | | 80.5 | 28.4 | 18.0 | 13.5 | .5 | ... | 54.5 | 17.3 | 39.3 | | | | | |
| Indoor | 44.5 | 58.5 | 54.0 | 7.5 | 60.5 | 45.0 | 25.5 | 58.5 | 42.5 | 19.0 | 77.0 | 44.5 | 30.5 | 32.5 | 14.5 | | 81.5 | 31.8 | 9.0 | 15.0 | .5 | .5 | 84.0 | 21.8 | 35.8 | | | | | |
| Low | 66.0 | 52.5 | 54.0 | 66.0 | 72.0 | 62.1 | 48.5 | 65.0 | 63.5 | 32.5 | 86.5 | 61.2 | 47.0 | 55.0 | 54.5 | 3.0 | 78.0 | 47.5 | 8.0 | 8.5 | 1.0 | | 31.5 | 9.8 | 45.2 | | | | | |
| **Average** | 56.8 | 45.7 | 58.2 | 27.0 | 69.0 | 51.3 | 47.7 | 64.8 | 56.5 | 32.8 | 81.8 | 56.7 | 31.7 | 42.5 | 24.3 | 1.0 | 80.0 | 35.9 | 11.7 | 12.3 | .7 | .2 | 56.7 | 16.3 | 40.1 | 37.0 | 41.3 | 34.9 | 15.2 | 71.9 |
| **Lawrence, Kans.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 38.5 | 4.0 | .5 | 1.5 | 64.0 | 21.7 | .5 | ... | | | 81.0 | 16.3 | | ... | ... | | 39.5 | 7.9 | ... | ... | ... | ... | ... | ... | 11.5 | | | | | |
| Indoor | 29.0 | 36.0 | 16.5 | 2.0 | 69.0 | 30.5 | 14.0 | 27.0 | ... | | 73.5 | 22.9 | 1.0 | | ... | | 71.5 | 14.5 | | ... | ... | | 26.5 | 5.3 | 18.3 | | | | | |
| Low | | 1.5 | ... | ... | 69.0 | 14.1 | ... | ... | ... | | 79.5 | 15.9 | | | | ... | 74.5 | 14.9 | ... | ... | | ... | 24.5 | 4.9 | 12.5 | | | | | |
| **Average** | 22.5 | 13.8 | 5.7 | 1.2 | 67.3 | 22.1 | 4.8 | 9.0 | ... | ... | 78.0 | 18.4 | .2 | | | | 61.8 | 12.4 | | ... | ... | ... | 17.0 | 3.4 | 14.1 | 6.9 | 5.5 | 1.4 | .3 | 56.0 |
| **New Haven, Conn.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 38.5 | 36.0 | 41.5 | 8.0 | 77.0 | 40.2 | 14.0 | 7.5 | 1.0 | .5 | 80.0 | 20.6 | | 1.0 | | | 78.5 | 15.9 | | | ... | ... | 45.5 | 9.1 | 21.5 | | | | | |
| Indoor | 62.0 | 63.5 | 67.0 | 69.0 | 68.0 | 65.0 | 69.5 | 58.0 | 51.5 | 19.5 | 73.0 | 54.3 | 19.0 | 32.5 | 1.0 | ... | 78.0 | 26.1 | | ... | ... | ... | 45.5 | 9.1 | 38.9 | | | | | |
| Low | 10.0 | 20.5 | 1.5 | ... | 72.0 | 22.0 | ... | | | .5 | 65.0 | 13.1 | | | ... | .5 | 75.5 | 15.2 | | ... | | | | | 12.6 | | | | | |
| **Average** | 36.8 | 42.0 | 30.7 | 25.7 | 72.3 | 42.7 | 27.8 | 21.8 | 17.5 | 6.8 | 72.7 | 29.3 | 6.3 | 11.2 | .3 | .2 | 77.3 | 19.1 | ... | ... | | ... | 30.3 | 6.1 | 24.3 | 17.7 | 18.8 | 13.6 | 8.2 | 63.2 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Pikes Peak, Colo.** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 52.0 | 74.5 | 63.0 | 43.0 | 79.5 | 62.4 | 71.5 | 79.0 | 51.5 | 71.0 | 85.5 | 77.7 | 71.0 | 74.5 | 68.0 | 66.0 | 81.5 | 72.2 | 56.0 | 68.0 | 57.0 | 59.0 | 68.5 | 61.7 | 67.0 | | | | | |
| Indoor | 73.0 | 73.5 | 62.5 | 72.0 | 92.5 | 74.7 | 69.0 | 77.5 | 65.0 | 60.0 | 77.0 | 69.7 | 73.0 | 71.5 | 69.0 | 74.0 | 81.5 | 73.8 | 46.0 | 66.0 | 51.5 | 31.5 | 69.0 | 52.8 | 67.8 | | | | | |
| Low | 36.0 | 39.0 | 40.0 | 13.0 | 80.5 | 41.7 | 8.5 | 3.0 | 7.5 | | 81.0 | 20.0 | 6.0 | 3.0 | 1.5 | | 72.0 | 16.5 | ... | ... | ... | ... | 72.0 | 14.4 | 23.2 | | | | | |
| Average | 53.7 | 62.3 | 55.2 | 42.7 | 84.2 | 59.6 | 49.7 | 53.2 | 41.3 | 43.7 | 81.2 | 53.8 | 50.0 | 49.7 | 46.2 | 46.7 | 78.3 | 54.2 | 24.0 | 44.7 | 36.2 | 30.2 | 69.8 | 43.0 | 52.7 | 46.8 | 52.5 | 44.7 | 40.8 | 78.4 |
| **Pocatello, Idaho:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 73.5 | 24.0 | 37.5 | 53.5 | 49.5 | 47.6 | 68.5 | 74.0 | 66.0 | 73.5 | 84.5 | 73.3 | 55.0 | 57.5 | 65.5 | 65.5 | 73.5 | 63.4 | 48.5 | 54.5 | 20.5 | 39.5 | 64.5 | 45.5 | 57.5 | | | | | |
| Indoor | 64.0 | 74.5 | 78.5 | 61.5 | 61.0 | 67.9 | 69.0 | 62.5 | 73.0 | 64.5 | 78.5 | 69.5 | 56.0 | 44.0 | 59.5 | 57.0 | 63.0 | 55.9 | 2.5 | 1.5 | 5.0 | 4.0 | 57.5 | 14.1 | 51.9 | | | | | |
| Low | 30.5 | 38.5 | 29.5 | 9.0 | 57.0 | 32.9 | 43.0 | 39.0 | 41.5 | 1.5 | 85.0 | 42.0 | 16.5 | 14.0 | 7.0 | | 78.5 | 23.2 | .5 | 1.5 | ... | ... | 75.5 | 15.3 | 28.4 | | | | | |
| Average | 56.0 | 45.7 | 48.5 | 42.3 | 55.8 | 49.5 | 60.2 | 58.5 | 60.2 | 40.5 | 82.7 | 61.6 | 42.5 | 38.5 | 44.0 | 40.8 | 71.7 | 47.5 | 17.2 | 10.2 | 8.5 | 14.5 | 65.8 | 25.0 | 45.9 | 44.0 | 40.5 | 40.3 | 35.8 | 69.0 |
| **State College, Pa.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 19.0 | 43.5 | 25.0 | 43.0 | 66.0 | 39.3 | 29.5 | 39.5 | 14.5 | 1.0 | 77.5 | 32.4 | 7.0 | 5.0 | .5 | | 68.0 | 16.1 | .5 | ... | ... | ... | 80.0 | 16.1 | 26.0 | | | | | |
| Indoor | 55.5 | 52.0 | 64.5 | 54.5 | 74.0 | 60.1 | 53.0 | 63.5 | 25.5 | 16.0 | 80.0 | 47.6 | 21.0 | 23.5 | 5.0 | | 65.5 | 23.0 | ... | ... | ... | | 47.0 | 9.4 | 35.0 | | | | | |
| Low | 20.5 | 66.5 | 22.0 | 26.0 | 55.0 | 30.8 | .5 | 3.0 | | ... | 84.0 | 17.5 | (a) | (a) | (a) | (a) | (a) | (a) | ... | ... | ... | | 84.0 | 16.8 | b24.7 | | | | | |
| Average | 34.7 | 54.0 | 37.2 | 41.2 | 65.0 | 46.4 | 27.7 | 35.3 | 13.3 | 5.7 | 80.5 | 32.5 | 14.0 | 14.3 | 2.8 | | 66.8 | 19.6 | .2 | ... | | ... | 70.3 | 14.1 | 28.2 | 19.2 | 25.9 | 17.8 | 11.7 | 70.7 |
| **Warsaw, Ky.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 36.0 | 45.0 | 20.0 | 5.5 | 58.5 | 33.0 | 2.5 | 4.5 | ... | | 76.0 | 16.6 | | | | | 22.5 | 4.5 | ... | ... | ... | ... | 57.0 | 11.4 | 16.4 | | | | | |
| Indoor | 33.0 | 41.5 | 14.0 | 1.0 | 67.0 | 31.3 | 4.5 | 4.5 | | .5 | 66.0 | 15.1 | ... | | | | 70.5 | 15.9 | ... | | ... | | 61.5 | 12.3 | 18.7 | | | | | |
| Low | 23.0 | 28.0 | 5.5 | 3.5 | 68.5 | 25.7 | ... | 13.5 | | | 61.5 | 15.0 | | | ... | | 72.0 | 14.4 | | ... | | ... | 65.5 | 13.1 | 17.1 | | | | | |
| Average | 30.7 | 38.2 | 13.2 | 3.3 | 64.7 | 30.0 | 2.6 | 7.5 | | .2 | 62.8 | 15.6 | | | | | 58.0 | 11.6 | | ... | ... | ... | 61.3 | 12.3 | 17.4 | 8.2 | 11.4 | 3.4 | .8 | 67.0 |
| **Waukegan, Ill.** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 53.5 | 35.0 | 61.0 | 32.0 | 71.0 | 50.5 | 45.0 | 54.5 | 13.0 | 4.5 | 73.0 | 38.0 | 3.0 | 18.5 | 3.5 | | 88.5 | 24.7 | | | | ... | 76.5 | 15.1 | 32.1 | | | | | |
| Indoor | 68.5 | 43.5 | 38.5 | 19.5 | 53.5 | 44.7 | 40.0 | 51.5 | 24.0 | 16.5 | 77.0 | 41.8 | 16.5 | 16.0 | | | 67.0 | 19.4 | | | | .5 | 59.0 | 11.0 | 29.6 | | | | | |
| Low | 20.5 | 38.5 | 11.0 | 3.0 | 58.5 | 26.5 | 31.0 | 21.5 | 1.5 | | 65.5 | 20.3 | .5 | 1.5 | .5 | | 87.5 | 18.0 | | | | | 73.5 | 14.7 | 19.8 | | | | | |
| Average | 47.5 | 39.0 | 36.8 | 18.2 | 61.0 | 40.5 | 32.0 | 42.5 | 13.5 | [illegible] | 71.8 | 33.4 | 6.7 | 15.3 | 1.3 | | 81.0 | 20.9 | | | | | 69.7 | 14.0 | 27.2 | 21.6 | 24.2 | 12.9 | 6.4 | 70.0 |
| Average for all storage points | 43.1 | 46.6 | 37.8 | [illegible] | 67.5 | [illegible] | 34.6 | 39.1 | [illegible] | [illegible] | [illegible] | 30.8 | 20.9 | 27.4 | [illegible] | [illegible] | [illegible] | [illegible] | 6.9 | 8.1 | 5.6 | 4.7 | 58.0 | 16.0 | | | | | | |

a Not sown.

b Average for 3 years.

TABLE V.—*Germination percentages of seed of Pseudotsuga taxifolia at various storage points*

[Leaders indicate "no germination"]

| Storage point and temperature condition. | 1910, 30-day test. | | | | | | 1911, 34-day test. | | | | | | 1912, 34-day test. | | | | | | 1914, 34-day test | | | | | | Average for all containers for all 4 years. | Average for each container at all 3 temperatures for all 4 years | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. |
| Ann Arbor, Mich.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 14.5 | 15.0 | 1.0 | ... | 19.5 | 10.0 | 9.0 | 15.0 | ... | ... | 44.5 | 13.7 | 0.5 | 0.5 | ... | ... | 39.5 | 8.1 | ... | ... | ... | ... | 47.5 | 9.5 | 10.3 | | | | | |
| Indoor | 24.0 | 34.0 | 22.5 | 18.0 | 37.0 | 27.1 | 25.5 | 21.0 | 2.0 | ... | 41.0 | 17.9 | .5 | 5.0 | 3.0 | ... | 44.5 | 10.6 | ... | ... | ... | ... | 49.0 | 9.8 | 16.4 | | | | | |
| Low | 20.0 | 36.0 | 12.5 | .5 | 48.0 | 23.4 | 14.0 | 32.5 | .5 | | 45.5 | 18.5 | 2.0 | 3.5 | ... | ... | 53.5 | 11.8 | ... | .5 | | ... | 30.0 | 6.1 | 15.0 | | | | | |
| Average | 19.5 | 28.3 | 12.0 | 6.2 | 34.8 | 20.2 | 16.2 | 22.8 | .8 | ... | 43.7 | 16.7 | 1.0 | 3.0 | 1.0 | ... | 45.8 | 10.2 | ... | .2 | ... | ... | 42.2 | 8.5 | 13.9 | 9.2 | 13.6 | 3.4 | 1.6 | 41.6 |
| Dundee, Ill.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 2.5 | 9.0 | .5 | ... | 36.5 | 9.7 | 2.0 | ... | ... | 49.5 | 45.0 | 19.3 | ... | ... | ... | ... | 48.0 | 9.6 | ... | ... | ... | ... | 50.0 | 10.0 | 12.2 | | | | | |
| Indoor | 6.0 | 15.5 | 1.0 | ... | 51.0 | 14.7 | ... | 2.0 | ... | ... | 38.5 | 8.1 | ... | ... | ... | ... | 49.5 | 9.9 | ... | ... | ... | ... | 43.0 | 8.6 | 10.3 | | | | | |
| Low | 2.0 | 12.5 | ... | ... | 59.5 | 14.8 | ... | 1.0 | ... | ... | 69.0 | 14.0 | ... | ... | ... | ... | 51.0 | 10.2 | 0.5 | ... | ... | | 48.0 | 9.7 | 12.2 | | | | | |
| Average | 3.5 | 12.3 | .5 | ... | 49.0 | 13.1 | .7 | 1.0 | ... | 16.5 | 50.8 | 13.8 | ... | ... | ... | ... | 49.5 | 9.9 | .2 | ... | ... | ... | 47.0 | 9.4 | 11.6 | 1.1 | 3.3 | .1 | 4.1 | 49.1 |
| Fort Bayard, N. Mex.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 29.0 | 34.5 | ... | ... | 55.5 | 23.8 | 25.5 | 46.0 | 19.0 | 5.0 | 54.5 | 30.0 | 13.5 | 10.5 | 7.5 | 1.0 | 40.5 | 14.6 | .5 | 9.5 | ... | ... | 35.5 | 9.1 | 19.4 | | | | | |
| Indoor | 32.5 | 40.5 | 43.0 | 37.5 | 45.5 | 39.8 | 33.5 | 40.0 | 42.5 | 34.5 | 44.0 | 39.3 | 21.0 | 23.5 | 17.5 | ... | 52.5 | 22.9 | .5 | ... | 0.5 | 4.0 | 44.5 | 9.9 | 28.0 | | | | | |
| Low | 34.0 | 35.0 | 37.5 | 18.0 | 56.0 | 36.1 | 38.0 | 26.5 | 44.5 | 8.0 | 53.0 | 34.0 | (a) | (a) | (a) | (a) | (a) | (a) | 15.5 | 14.5 | 19.0 | 1.0 | 54.5 | 20.9 | 30.3 | | | | | |
| Average | 31.8 | 36.7 | 26.8 | 18.5 | 52.3 | 33.2 | 33.0 | 37.5 | 35.3 | 15.8 | 50.5 | 34.4 | 17.3 | 17.0 | 12.5 | .5 | 46.5 | 18.8 | 5.5 | 8.0 | 6.5 | 1.7 | 44.8 | 13.3 | 24.9 | 21.9 | 24.8 | 20.3 | 9.1 | 48.5 |
| Halsey, Nebr.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 31.5 | 43.0 | 14.5 | 1.0 | 29.0 | 23.8 | 8.5 | 13.5 | 2.0 | ... | 50.0 | 14.8 | ... | .5 | ... | ... | 46.5 | 9.4 | ... | ... | ... | ... | 11.5 | 2.3 | 12.6 | | | | | |
| Indoor | 39.0 | 39.0 | 36.0 | 5.5 | 42.5 | 32.4 | 8.5 | 18.0 | 6.5 | ... | 54.5 | 17.5 | 2.5 | 1.0 | .5 | ... | 51.5 | 11.1 | ... | ... | ... | ... | 25.5 | 5.1 | 16.5 | | | | | |
| Low | 14.5 | 27.0 | 1.0 | ... | 45.5 | 17.6 | .5 | 5.0 | ... | ... | 72.0 | 15.5 | ... | ... | ... | ... | 31.5 | 6.3 | ... | ... | ... | ... | 28.0 | 5.6 | 11.3 | | | | | |
| Average | 28.3 | 36.3 | 17.2 | 2.2 | 39.0 | 24.6 | 5.8 | 12.2 | 2.8 | ... | 58.8 | 15.9 | .8 | .5 | .2 | ... | 43.2 | 8.9 | ... | ... | ... | ... | 21.7 | 4.3 | 13.4 | 8.7 | 12.2 | 5.0 | .6 | 40.7 |
| Ithaca, N. Y.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 26.0 | 27.5 | 28.0 | 4.0 | 50.0 | 27.1 | 22.0 | 27.5 | 2.5 | ... | 55.5 | 21.5 | 1.5 | 3.0 | ... | ... | 50.0 | 10.9 | ... | ... | ... | ... | 29.0 | 5.8 | 16.3 | | | | | |
| Indoor | 37.0 | 50.0 | 21.5 | .5 | 50.0 | 31.8 | 13.0 | 21.5 | 9.0 | ... | 54.5 | 19.6 | ... | 5.5 | ... | ... | 57.5 | 12.6 | ... | ... | ... | ... | 7.5 | 1.5 | 16.4 | | | | | |
| Low | 28.5 | 32.0 | 12.0 | ... | 54.5 | 25.4 | 3.0 | 5.5 | ... | ... | 45.5 | 10.8 | ... | ... | ... | ... | 58.5 | 11.7 | (a) | (a) | (a) | (a) | (a) | (a) | [b]16.0 | | | | | |
| Average | 30.5 | 36.5 | 20.5 | 1.5 | 51.5 | 28.1 | 12.7 | 18.2 | 3.8 | ... | 51.8 | 17.3 | .5 | 2.8 | ... | ... | 55.3 | 11.7 | ... | ... | ... | ... | 18.3 | 3.7 | 15.2 | 10.9 | 14.4 | 6.1 | .4 | 44.2 |
| Lake Clear Junction, N. Y.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 43.5 | 46.5 | 42.5 | 2.0 | 46.5 | 36.2 | 40.0 | 37.5 | 18.0 | . | 56.5 | 30.4 | 17.5 | 19.0 | 3.5 | | 56.0 | 19.2 | ... | ... | ... | .5 | 39.0 | 7.9 | 23.4 | | | | | |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Indoor | 37.5 | 45.5 | 41.5 | 6.0 | 55.5 | 37.2 | 14.0 | 32.0 | 20.0 | 2.5 | 60.0 | 25.7 | 5.5 | 12.5 | .5 | ... | 35.5 | 10.8 | ... | ... | ... | ... | 35.0 | 7.0 | 20.2 | | | | | |
| Low | 42.5 | 41.0 | 31.0 | 30.0 | 53.0 | 39.5 | 41.0 | 37.0 | 31.5 | 24.0 | 52.0 | 37.1 | 5.5 | 25.0 | 3.0 | ... | 42.5 | 15.2 | ... | 1.5 | ... | ... | 13.5 | 3.0 | 23.7 | | | | | |
| Average | 41.2 | 44.3 | 38.3 | 12.7 | 51.7 | 37.6 | 31.7 | 35.5 | 23.2 | 8.8 | 56.2 | 31.1 | 9.5 | 18.8 | 2.3 | ... | 44.7 | 15.1 | ... | .5 | ... | .2 | 19.2 | 6.0 | 22.4 | 20.6 | 24.8 | 16.0 | 5.4 | 45.4 |
| Lawrence, Kans.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 11.5 | 6.0 | ... | ... | 53.5 | 14.2 | ... | ... | ... | ... | 63.0 | 12.6 | ... | | | ... | 32.0 | 6.4 | ... | ... | ... | ... | | | 8.3 | | | | | |
| Indoor | 14.0 | 20.0 | ... | ... | 47.0 | 16.2 | 1.5 | ... | ... | ... | 55.0 | 11.3 | ... | ... | ... | ... | 54.0 | 10.8 | ... | ... | ... | ... | 6.5 | 1.3 | 9.9 | | | | | |
| Low | ... | .5 | ... | ... | 51.5 | 10.3 | 5.0 | ... | ... | .5 | 53.0 | 11.7 | ... | ... | ... | ... | 60.0 | 12.0 | ... | ... | ... | ... | 32.0 | 6.4 | 10.1 | | | | | |
| Average | 8.5 | 8.8 | ... | ... | 50.7 | 13.0 | 2.2 | ... | ... | .2 | 57.0 | 11.9 | ... | ... | ... | ... | 48.7 | 9.7 | ... | ... | ... | ... | 12.8 | 2.6 | 9.4 | 2.7 | 2.2 | .0 | .05 | 42.3 |
| New Haven, Conn.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 7.5 | 12.5 | ... | ... | 47.0 | 13.4 | ... | ... | ... | ... | 51.0 | 10.2 | ... | ... | ... | ... | 52.5 | 10.5 | ... | ... | ... | ... | 40.0 | 8.0 | 10.5 | | | | | |
| Indoor | 37.0 | 39.5 | 30.5 | 6.0 | 46.5 | 31.9 | 9.5 | 17.5 | ... | .5 | 44.0 | 14.3 | ... | 1.0 | ... | ... | 35.0 | 7.2 | ... | ... | ... | ... | 12.0 | 2.4 | 14.0 | | | | | |
| Low | 2.5 | 6.0 | .5 | .1 | 42.5 | 10.3 | ... | ... | ... | ... | 42.5 | 8.5 | ... | ... | ... | ... | 51.0 | 10.2 | ... | ... | ... | ... | 37.0 | 7.4 | 9.1 | | | | | |
| Average | 15.7 | 19.3 | 10.3 | 2.0 | 45.3 | 18.5 | 3.2 | 5.8 | ... | .2 | 45.8 | 11.0 | ... | .2 | ... | ... | 46.2 | 9.3 | ... | ... | ... | ... | 29.7 | 5.9 | 11.2 | 4.7 | 6.3 | 2.6 | .6 | 41.8 |
| Pikes Peak, Colo.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 36.5 | 37.5 | 27.5 | ... | 51.5 | 30.6 | 35.5 | 46.5 | 42.0 | 2.0 | 49.0 | 35.0 | 34.5 | 35.0 | 41.0 | 4.0 | 49.0 | 32.7 | 4.0 | 6.0 | .5 | ... | 31.0 | 8.3 | 26.7 | | | | | |
| Indoor | 42.0 | 44.0 | 50.0 | 23.5 | 55.5 | 43.0 | 36.0 | 32.0 | 54.5 | 4.5 | 62.0 | 37.8 | 23.0 | 23.0 | 23.5 | 11.0 | 46.0 | 25.3 | 5.0 | 11.0 | 6.5 | 5.0 | 40.5 | 13.6 | 29.9 | | | | | |
| Low | 8.0 | 6.5 | 5.5 | 34.5 | 34.0 | 17.7 | ... | ... | ... | ... | 56.5 | 11.3 | ... | ... | ... | ... | 33.5 | 6.7 | ... | ... | ... | ... | 29.5 | 5.9 | 10.4 | | | | | |
| Average | 28.8 | 29.3 | 27.7 | 19.3 | 47.0 | 30.4 | 23.8 | 26.2 | 32.2 | 2.2 | 55.8 | 28.0 | 19.2 | 19.3 | 21.5 | 5.0 | 42.8 | 21.6 | 3.0 | 5.7 | 2.3 | 1.7 | 33.7 | 9.3 | 22.3 | 18.7 | 20.1 | 20.9 | 7.0 | 44.8 |
| Pocatello, Idaho: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 43.0 | 35.0 | 42.0 | 37.0 | 49.5 | 41.3 | 32.5 | 42.5 | 50.5 | 28.0 | 59.0 | 42.5 | 22.0 | 17.5 | 30.0 | 18.0 | 35.0 | 24.5 | 1.5 | 3.0 | .5 | .5 | 28.0 | 6.7 | 28.8 | | | | | |
| Indoor | 36.0 | 46.0 | 38.5 | 49.0 | 49.5 | 43.8 | 20.5 | 16.0 | 16.0 | .5 | 40.5 | 18.7 | 4.0 | 7.0 | 3.0 | 1.0 | 35.0 | 10.0 | | | | | 10.0 | 6.0 | 19.0 | | | | | |
| Low | 5.5 | 6.5 | 2.5 | .5 | 55.0 | 14.0 | 3.0 | 20.0 | 1.0 | ... | 54.5 | 15.7 | ... | .5 | ... | ... | 48.0 | 9.7 | ... | | | | 30.5 | 7.3 | 11.7 | | | | | |
| Average | 28.2 | 29.2 | 27.7 | 28.8 | 51.3 | 33.0 | 18.7 | 26.2 | 22.5 | 9.5 | 51.3 | 25.6 | 8.7 | 8.5 | 11.0 | 6.3 | 39.0 | 14.7 | .5 | 1.0 | .2 | .2 | 31.5 | 6.7 | 20.0 | 14.0 | 16.2 | 15.4 | 11.2 | 43.3 |
| State College, Pa.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 18.5 | 23.0 | 14.0 | 6.5 | 50.5 | 22.5 | 6.5 | 14.5 | .5 | | 43.5 | 13.0 | ... | ... | ... | | 51.5 | 10.3 | ... | ... | | | 34.5 | 6.9 | 13.2 | | | | | |
| Indoor | 27.0 | 29.5 | 16.5 | 1.0 | 52.5 | 25.3 | 11.5 | 9.5 | 6.0 | | 38.0 | 13.0 | ... | ... | ... | ... | 40.5 | 8.1 | | | | | 10.0 | 3.1 | 12.4 | | | | | |
| Low | 8.0 | 32.5 | 6.5 | .5 | 55.5 | 20.6 | ... | | ... | .5 | 57.5 | 11.6 | (a) | (a) | (a) | (a) | (a) | (a) | ... | | | | 22.5 | 4.5 | [b]12.[illegible] | | | | | |
| Average | 17.8 | 28.3 | 12.3 | 2.7 | 52.8 | 22.8 | 6.0 | 8.0 | 2.2 | .2 | 46.3 | 12.5 | ... | | ... | | 40.0 | 9.2 | ... | | | ... | 24.3 | 4.8 | 12.4 | 6.0 | 9.1 | 3.6 | .7 | 42.4 |
| Warsaw, Ky.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 8.5 | 16.0 | ... | ... | 41.5 | 13.2 | ... | | | ... | 51.0 | 10.2 | | ... | | | 39.5 | 7.9 | | | | | 42.5 | 8.5 | 10.0 | | | | | |
| Indoor | 12.0 | 15.5 | 1.5 | ... | 52.0 | 16.2 | | | | . | 50.0 | 11.2 | | | | . | 40.5 | 8.1 | | | | | 29.0 | 5.5 | 10.1 | | | | | |
| Low | 5.0 | 15.5 | 1.0 | ... | 37.5 | 11.8 | .5 | ... | | | 57.5 | 11.0 | | | | . | 45.0 | 9.0 | | | | | 18.0 | 3.0 | 9.0 | | | | | |
| Average | 8.5 | 15.7 | .8 | | 43.7 | 13.7 | .2 | | | | 54.8 | 11.0 | | | | | 41.7 | 8.3 | | | | | 29.8 | 6.0 | 9.8 | 2.2 | 3.9 | .2 | | 42.5 |
| Waukegan, Ill.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 23.0 | 29.0 | 14.5 | ... | 45.5 | 22.4 | 10.5 | 20.5 | 15.0 | 5.0 | 47.0 | 18.8 | .5 | | | | 51.0 | 10.3 | | | | | 7.0 | 1.4 | 15.2 | | | | | |
| Indoor | 28.5 | 29.0 | 18.0 | ... | 54.0 | 25.9 | 13.5 | 20.0 | 3.0 | | 43.5 | 16.0 | . | | . | | 54.0 | 10.8 | ... | | | | 31.0 | 6.[illegible] | 14.7 | | | | | |
| Low | 4.5 | 4.0 | | ... | 55.5 | 12.8 | | | .5 | | 58.5 | 11.8 | . | | . | | 50.0 | 10.0 | | | | | 13.5 | 10.7 | 11.3 | | | | | |
| Average | 18.7 | 20.7 | 10.8 | ... | 51.7 | 20.4 | 8.0 | 13.5 | 6.2 | .2 | 49.7 | 15.5 | .3 | | . | | 51.7 | 10.4 | . | | | | 30.5 | 6.1 | 13.1 | 6.7 | 8.6 | 4.2 | .05 | 45.9 |
| Average for all storage points | 31.0 | 26.6 | 13.8 | 7.2 | 47.8 | 23.8 | 12.5 | 15.9 | 9.9 | 4.1 | 51.7 | 18.8 | 4.2 | 5.2 | 3.6 | .9 | 46.3 | 12.0 | .7 | 1.2 | .7 | 1 | 30.[illegible] | 6.7 | | | | | | |

a Not sown.

b Average for 1 years

TABLE VI.—*Germination percentages of seed of Pinus ponderosa at various storage points*

[Leaders indicate "no germination"]

| Storage point and temperature condition. | 1910, 103 day test. | | | | | | 1911, 117 day test. | | | | | | 1912, 117-day test. | | | | | | 1914, 113 day test. | | | | | | Average for all containers for all 4 years. | Average for each container at all 3 temperatures for all 4 years. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. |
| **Ann Arbor, Mich.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 76.5 | 81.5 | 72.5 | 80.5 | 76.0 | 77.4 | 49.5 | 42.0 | 53.5 | 44.5 | 65.0 | 50.9 | 63.0 | 68.0 | 57.0 | 64.0 | 79.5 | 66.3 | 41.0 | 65.5 | 38.0 | 25.0 | 72.0 | 48.3 | 60.7 | | | | | |
| Indoor | 78.0 | 73.5 | 82.5 | 81.0 | 80.5 | 79.1 | 60.5 | 60.5 | 61.5 | 67.5 | 68.0 | 63.6 | 68.0 | 68.0 | 75.5 | 67.0 | 78.5 | 71.4 | 79.5 | 68.0 | 49.0 | 55.5 | 74.0 | 65.2 | 69.8 | | | | | |
| Low | 74.0 | 79.0 | 71.0 | 80.0 | 77.5 | 76.3 | 62.5 | 56.5 | 66.0 | 59.0 | 73.0 | 63.4 | 68.0 | 71.0 | 73.5 | 57.0 | 70.0 | 67.9 | 80.0 | 72.0 | 81.0 | 59.0 | 63.0 | 71.0 | 69.7 | | | | | |
| Average | 76.2 | 78.0 | 75.3 | 80.5 | 78.0 | 77.6 | 57.5 | 53.0 | 60.3 | 57.0 | 68.7 | 59.3 | 66.3 | 69.0 | 68.7 | 62.7 | 75.8 | 68.5 | 66.8 | 68.5 | 56.0 | 46.5 | 69.7 | 61.5 | 66.7 | 66.7 | 67.1 | 65.1 | 61.7 | 73.0 |
| **Dundee, Ill.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 72.5 | 77.5 | 75.0 | 73.0 | 75.5 | 74.7 | 73.5 | 71.5 | 63.5 | 76.5 | 78.5 | 72.7 | 49.5 | 55.0 | 41.5 | 35.5 | 74.5 | 51.2 | 7.5 | 40.0 | 3.5 | 2.0 | 67.0 | 24.0 | 55.7 | | | | | |
| Indoor | 78.0 | 77.5 | 74.0 | 77.0 | 82.0 | 77.7 | 75.0 | 60.5 | 66.0 | 59.0 | 74.0 | 66.9 | 48.0 | 50.5 | 51.5 | 41.5 | 80.5 | 54.4 | 3.5 | 28.0 | 5.5 | 1.0 | 82.0 | 24.0 | 55.8 | | | | | |
| Low | 73.0 | 72.0 | 72.5 | 73.0 | 75.5 | 73.2 | 62.0 | 77.5 | 76.5 | 65.0 | 69.5 | 70.1 | 42.0 | 61.5 | 30.5 | 48.0 | 82.0 | 52.8 | 6.0 | 16.0 | 2.5 | .5 | 74.5 | 19.9 | 54.0 | | | | | |
| Average | 74.5 | 75.7 | 73.8 | 74.3 | 77.7 | 75.2 | 70.2 | 69.8 | 68.7 | 66.8 | 74.0 | 69.9 | 46.5 | 55.7 | 41.2 | 41.7 | 79.0 | 52.8 | 5.7 | 28.0 | 3.8 | 1.2 | 74.5 | 22.6 | 55.1 | 49.2 | 57.3 | 46.9 | 46.0 | 76.3 |
| **Fort Bayard, N. Mex.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 81.0 | 80.0 | 79.5 | 58.0 | 83.5 | 76.4 | 75.0 | 77.0 | 73.0 | 73.0 | 77.5 | 75.1 | 65.5 | 67.0 | 66.0 | 74.5 | 79.0 | 70.4 | 75.0 | 72.5 | 55.0 | 67.0 | 83.5 | 70.6 | 73.1 | | | | | |
| Indoor | 84.5 | 82.5 | 79.0 | 81.5 | 80.5 | 81.6 | 76.5 | 81.0 | 80.0 | 75.0 | 80.0 | 78.5 | 67.0 | 70.5 | 64.0 | 72.0 | 72.0 | 69.1 | 73.5 | 76.0 | 78.5 | 73.0 | 75.0 | 75.2 | 76.1 | | | | | |
| Low | 76.0 | 69.5 | 80.0 | 76.0 | 79.5 | 76.2 | 70.0 | 75.0 | 72.5 | 77.5 | 64.0 | 71.8 | (a) | (a) | (a) | (a) | (a) | (a) | 72.5 | 60.5 | 68.0 | 70.0 | 81.5 | 72.3 | [b]73.4 | | | | | |
| Average | 80.5 | 77.3 | 79.5 | 71.8 | 81.2 | 78.1 | 73.8 | 77.7 | 75.2 | 75.2 | 73.8 | 75.1 | 66.3 | 68.8 | 65.0 | 73.3 | 75.5 | 69.8 | 73.7 | 72.7 | 67.2 | 70.0 | 80.0 | 72.7 | 73.9 | 73.6 | 74.1 | 71.7 | 72.6 | 77.6 |
| **Halsey, Nebr.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 71.5 | 79.0 | 72.0 | 67.0 | 74.0 | 72.7 | 76.0 | 78.0 | 78.0 | 73.5 | 79.0 | 76.9 | 67.0 | 68.0 | 62.5 | 64.0 | 54.0 | 63.1 | 67.0 | 63.0 | 62.0 | 15.0 | 77.5 | 56.9 | 67.4 | | | | | |
| Indoor | 72.5 | 74.0 | 79.0 | 80.0 | 76.0 | 76.3 | 75.0 | 74.0 | 79.0 | 63.0 | 76.5 | 73.5 | 69.0 | 39.0 | 73.0 | 70.5 | 78.5 | 66.0 | 64.0 | 66.0 | 72.5 | 58.0 | 76.5 | 67.4 | 70.8 | | | | | |
| Low | 71.0 | 69.0 | 76.0 | 68.5 | 78.0 | 72.5 | 61.5 | 72.5 | 67.0 | 54.5 | 80.5 | 67.2 | 61.0 | 62.0 | 56.0 | 33.5 | 66.5 | 55.8 | 60.5 | 62.0 | 41.0 | 48.5 | 75.0 | 57.4 | 63.2 | | | | | |
| Average | 71.7 | 74.0 | 75.7 | 71.8 | 76.0 | 73.8 | 70.8 | 74.8 | 74.7 | 63.7 | 78.7 | 72.5 | 65.7 | 56.3 | 63.8 | 56.0 | 66.3 | 61.6 | 63.8 | 63.7 | 58.5 | 40.5 | 76.3 | 60.6 | 67.1 | 68.0 | 67.2 | 68.2 | 58.0 | 74.6 |
| **Ithaca, N. Y.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 75.5 | 68.5 | 74.5 | 76.0 | 74.5 | 73.8 | 70.0 | 73.5 | 71.0 | 66.0 | 71.5 | 70.4 | 69.0 | 65.0 | 66.5 | 53.0 | 72.0 | 65.1 | 60.5 | 62.5 | 62.0 | 49.5 | 74.5 | 61.8 | 67.8 | | | | | |
| Indoor | 63.5 | 79.5 | 74.5 | 81.0 | 68.5 | 73.4 | 73.0 | 75.0 | 71.5 | 69.0 | 76.5 | 73.0 | 71.0 | 73.5 | 77.0 | 61.5 | 71.5 | 70.9 | 65.0 | 58.0 | 66.0 | 59.0 | 78.0 | 65.2 | 70.6 | | | | | |
| Low | 85.0 | 79.0 | 83.5 | 77.5 | 70.5 | 79.1 | 53.5 | 60.5 | 58.5 | 52.0 | 69.5 | 58.8 | 54.0 | 58.5 | 48.0 | 48.5 | 83.5 | 58.5 | (a) | (a) | (a) | (a) | (a) | (a) | [b]65.5 | | | | | |
| Average | 74.7 | 75.7 | 77.5 | 78.2 | 71.2 | 75.5 | 65.5 | 69.7 | 67.0 | 62.3 | 72.5 | 67.4 | 64.7 | 65.7 | 63.8 | 54.3 | 75.5 | 64.8 | 62.8 | 60.3 | 64.0 | 54.3 | 76.3 | 63.5 | 67.8 | 66.9 | 67.8 | 68.1 | 62.3 | 73.9 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lake Clear Junction, N. Y.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Fluctuating** | 74.5 | 79.5 | 79.5 | 70.5 | 80.5 | 76.9 | 71.5 | 69.5 | 67.0 | 67.5 | 70.0 | 69.1 | 72.0 | 71.5 | 75.0 | 70.0 | 82.5 | 74.0 | 72.0 | 65.0 | 72.5 | 60.5 | 84.0 | 70.8 | 72.7 | | | | | |
| **Indoor** | 79.0 | 70.5 | 80.0 | 67.5 | 68.5 | 73.1 | 59.0 | 69.5 | 66.5 | 45.0 | 68.0 | 61.6 | 60.0 | 61.0 | 61.0 | 54.0 | 83.0 | 63.8 | 58.0 | 68.5 | 48.0 | 24.0 | 72.0 | 54.1 | 63.2 | | | | | |
| **Low** | 76.0 | 76.0 | 80.0 | 73.0 | 82.5 | 77.5 | 70.5 | 69.5 | 70.5 | 66.5 | 73.5 | 70.1 | 72.0 | 75.0 | 68.5 | 73.0 | 74.0 | 72.5 | 77.5 | 68.0 | 74.5 | 61.5 | 71.0 | 70.5 | 72.7 | | | | | |
| **Average** | 76.5 | 75.3 | 79.8 | 70.3 | 77.2 | 73.8 | 67.0 | 69.5 | 68.0 | 59.7 | 70.5 | 66.9 | 68.0 | 60.2 | 67.8 | 65.7 | 79.8 | 70.1 | 69.2 | 67.2 | 65.0 | 48.7 | 75.7 | 65.2 | 69.5 | 70.2 | 70.3 | 70.2 | 61.1 | 75.8 |
| **Lawrence, Kans.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Fluctuating** | 78.5 | 74.0 | 76.5 | 77.0 | 79.0 | 72.0 | 71.0 | 39.5 | 52.0 | 34.5 | 79.0 | 55.2 | 53.0 | 35.5 | 53.0 | 11.0 | 74.5 | 45.1 | 8.0 | 4.5 | 5 | | 53.5 | 13.3 | 47.7 | | | | | |
| **Indoor** | 74.5 | 68.0 | 72.0 | 58.5 | 72.5 | 69.1 | 69.5 | 61.5 | 23.0 | 59.5 | 82.5 | 69.2 | 59.5 | 63.5 | 56.5 | 55.0 | 77.0 | 54.1 | 43.5 | 50.0 | 10.5 | 7.0 | 80.5 | 38.3 | 57.7 | | | | | |
| **Low** | 62.5 | 71.5 | 65.5 | 24.5 | 77.0 | 60.2 | 53.5 | 63.0 | 38.5 | 21.0 | 78.0 | 50.8 | 23.5 | 49.0 | 21.0 | 3.0 | 75.0 | 34.7 | 3.0 | 13.5 | 3.5 | 1.0 | 84.0 | 21.0 | 41.7 | | | | | |
| **Average** | 71.8 | 71.2 | 71.3 | 53.3 | 76.2 | 68.8 | 64.7 | 54.7 | 54.5 | 38.3 | 79.8 | 58.4 | 45.3 | 49.3 | 44.2 | 9.7 | 75.5 | 44.8 | 18.2 | 22.7 | 4.8 | 2.7 | 72.7 | 24.2 | 49.0 | 50.0 | 49.5 | 43.7 | 26.0 | 76.0 |
| **New Haven, Conn.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Fluctuating** | 68.0 | 78.5 | 70.5 | 68.5 | 78.0 | 72.7 | 50.5 | 50.5 | 53.0 | 31.5 | 67.5 | 50.6 | 23.0 | 33.0 | 17.0 | 4.0 | 72.0 | 29.8 | 4.5 | 6.0 | 7.0 | .5 | 67.5 | 17.1 | 42.6 | | | | | |
| **Indoor** | 76.5 | 78.5 | 76.0 | 76.0 | 80.0 | 77.4 | 60.5 | 61.0 | 57.5 | 59.5 | 77.0 | 63.1 | 74.0 | 70.0 | 70.0 | 64.5 | 71.0 | 69.9 | 62.0 | 69.5 | 31.0 | 24.0 | 75.5 | 52.4 | 65.7 | | | | | |
| **Low** | 69.5 | 74.5 | 66.5 | 78.5 | 76.0 | 73.0 | 7.5 | 8.5 | 8.5 | 22.0 | 72.0 | 23.7 | | . | ... | 1.0 | 80.5 | 36.3 | ... | . | | 5 | 74.5 | 35.0 | 32.0 | | | | | |
| **Average** | 71.3 | 77.2 | 71.0 | 74.3 | 78.0 | 74.4 | 39.5 | 40.0 | 39.7 | 37.7 | 72.2 | 45.8 | 32.3 | 34.3 | 29.0 | 23.3 | 74.5 | 38.7 | 22.2 | 25.2 | 12.7 | 8.3 | 72.5 | 28.2 | 46.8 | 41.3 | 44.2 | 38.7 | 35.9 | 74.1 |
| **Pikes Peak, Colo.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Fluctuating** | 78.0 | 78.5 | 81.5 | (a) | 73.5 | 77.9 | 37.5 | 40.0 | 61.0 | (a) | 69.0 | 51.9 | 64.5 | 64.5 | 61.0 | (a) | 73.5 | 65.9 | 71.5 | 72.5 | 77.5 | (a) | 79.5 | 75.2 | 67.2 | | | | | |
| **Indoor** | 79.5 | 74.5 | 80.0 | (a) | 76.5 | 77.6 | 64.0 | 67.0 | 72.0 | (a) | 63.0 | 66.5 | 64.5 | 72.5 | 72.0 | (a) | 83.0 | 73.0 | 66.5 | 77.0 | 79.5 | (a) | 73.9 | 74.0 | 72.8 | | | | | |
| **Low** | 69.5 | 67.0 | 67.0 | (a) | 83.5 | [illegible] | [illegible] | 18.0 | 17.0 | (a) | 67.0 | 20.3 | 20.0 | 17.0 | 10.5 | (a) | 76.5 | 31.0 | 10.0 | 9.5 | 8.5 | (a) | 64.0 | 23.0 | 38.7 | | | | | |
| **Average** | 75.7 | 73.3 | 76.2 | .. | 77.7 | 75.7 | 38.8 | 41.7 | 30.0 | .. | 66.3 | 40.2 | 40.7 | 53.3 | 47.8 | | 77.7 | 56.6 | 40.3 | 53.0 | 55.2 | . | 72.7 | 57.4 | 59.7 | 55.4 | 54.8 | 57.3 | (a) | 73.5 |
| **Pocatello, Idaho:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Fluctuating** | 87.5 | 71.5 | 74.0 | 74.0 | 82.0 | 76.8 | 63.0 | 65.0 | 70.0 | 78.5 | 69.5 | 69.2 | 60.0 | 71.0 | 68.0 | 62.0 | 67.0 | 66.0 | 70.0 | 70.5 | 71.5 | 73.5 | 66.5 | 70.8 | 70.7 | | | | | |
| **Indoor** | 69.0 | 80.0 | 81.0 | 67.5 | 75.5 | 74.6 | 70.5 | 72.5 | 67.0 | 58.5 | 65.5 | 66.8 | 70.5 | 70.5 | 62.0 | 67.0 | 67.0 | 67.6 | 47.5 | 59.0 | 53.5 | 43.5 | 79.5 | 56.6 | 66.4 | | | | | |
| **Low** | 60.0 | 75.5 | 85.0 | 61.5 | 86.5 | 75.9 | 58.0 | 51.5 | 40.0 | 37.0 | 44.0 | 47.3 | 60.0 | 59.5 | 51.5 | 51.5 | 78.5 | 60.2 | 71.0 | 59.5 | 67.5 | 60.0 | 78.5 | 67.3 | 62.7 | | | | | |
| **Average** | 73.5 | 75.7 | 80.0 | 68.3 | 81.3 | 75.8 | 63.8 | 63.0 | 61.0 | 58.0 | 59.7 | 61.1 | 63.5 | 69.0 | 60.5 | 58.5 | 71.5 | 64.6 | 62.8 | 63.7 | 64.2 | 59.0 | 74.8 | 64.9 | 66.6 | 65.9 | 67.8 | 66.4 | 61.0 | 71.8 |
| **State College, Pa.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Fluctuating** | 74.5 | 71.0 | 79.0 | (a) | 76.0 | 75.1 | 62.0 | 65.5 | 65.5 | (a) | 73.5 | 66.6 | 55.5 | 67.0 | 58.5 | (a) | 81.5 | 64.6 | 57.0 | 64.0 | 51.5 | (a) | 78.5 | 62.8 | 67.3 | | | | | |
| **Indoor** | 72.5 | 77.5 | 71.0 | (a) | 72.0 | 73.2 | 67.5 | 65.5 | 64.0 | (a) | 67.5 | 65.1 | 69.0 | 65.5 | 64.5 | (a) | 74.0 | 68.3 | 49.5 | 68.5 | 42.0 | (a) | 69.0 | 57.3 | 66.2 | | | | | |
| **Low** | 62.0 | 57.5 | 73.0 | (a) | 76.5 | 67.2 | 15.0 | 23.5 | 21.3 | (a) | 41.5 | 25.4 | (a) | (a) | (a) | (a) | (a) | (a) | 7.0 | 20.5 | 10.0 | (a) | 71.5 | 27.3 | [b]40.0 | | | | | |
| **Average** | 69.7 | 68.7 | 74.3 | | 74.9 | 71.8 | 48.2 | 51.5 | 50.3 | | 60.8 | 52.7 | 62.3 | 64.3 | 61.5 | | 77.8 | 66.5 | 37.8 | 51.0 | 34.5 | | 73.0 | 40.1 | 60.0 | 54.5 | 58.9 | 55.2 | (a) | 71.6 |
| **Warsaw, Ky.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Fluctuating** | 77.5 | 71.3 | 73.0 | (a) | 83.5 | 70.4 | 52.0 | 50.5 | 45.5 | (a) | 70.0 | 54.5 | 13.0 | 28.5 | 10.5 | (a) | 73.0 | 31.3 | . | 1.5 | | (a) | 70.5 | 18.0 | 45.0 | | | | | |
| **Indoor** | 79.5 | 75.5 | 75.5 | (a) | 78.4 | 77.2 | 55.0 | 65.0 | 49.5 | (a) | 61.5 | 51.8 | 27.5 | 47.5 | 17.0 | (a) | 66.5 | 39.6 | .5 | . | | (a) | 82.5 | 20.8 | 47.6 | | | | | |
| **Low** | 75.5 | 69.5 | 77.0 | (a) | 76.5 | 74.6 | 40.5 | 54.0 | 37.0 | (a) | 66.5 | 49.5 | 29.0 | 20.5 | 10.5 | (a) | 78.5 | 34.6 | 5 | | | (a) | 83.0 | 20.9 | 44.9 | | | | | |
| **Average** | 77.5 | 72.2 | 75.2 | . | 79.4 | 76.1 | 49.2 | 56.5 | 37.3 | .. | 66.0 | 52.3 | 20.3 | 35.2 | 12.7 | -- | 77.7 | 35.2 | 2 | 5 | | . | 78.7 | 19.9 | 45.8 | 36.8 | 41.1 | 31.3 | (a) | 74.2 |
| **Waukegan, Ill.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Fluctuating** | 77.5 | 72.0 | 69.5 | (a) | 81.5 | 75.1 | 79.0 | 71.5 | 75.0 | (a) | 68.5 | 73.5 | 67.0 | 65.5 | 77.5 | (a) | 82.5 | 73.1 | 55.0 | 63.0 | 49.5 | (a) | 71.5 | 59.8 | 70.4 | | | | | |
| **Indoor** | 77.5 | 91.0 | 78.5 | (a) | 78.5 | 79.9 | 74.0 | 68.5 | 68.5 | (a) | 77.5 | 72.1 | 66.0 | 69.0 | 70.0 | (a) | 77.0 | 70.5 | 57.0 | 56.5 | 43.5 | (a) | 78.5 | 61.4 | 71.0 | | | | | |
| **Low** | 74.0 | 74.0 | 63.5 | (a) | 81.5 | 73.2 | 55.5 | 64.5 | 60.0 | (a) | 75.5 | 63.9 | 48.5 | 71.0 | 56.0 | (a) | 69.5 | 61.8 | 2.0 | .5 | 1.0 | (a) | 70.0 | 20.9 | 55.0 | | | | | |
| **Average** | 74.3 | 79.0 | 70.5 | . | 80.5 | 77.1 | 69.5 | 68.2 | 67.8 | | 73.8 | 69.8 | 66.5 | 69.2 | 67.8 | | 76.3 | 68.5 | 38.0 | 43.1 | 31.7 | | 76.3 | 47.3 | 65.4 | 60.6 | 64.9 | 59.4 | (a) | 76.7 |
| **Average for all storage points** | 74.5 | 74.9 | 75.4 | 71.4 | 77.6 | 75.0 | 59.9 | 60.8 | 59.6 | 57.0 | 70.5 | 61.6 | 54.2 | 57.8 | 52.8 | 48.5 | 75.2 | 55.3 | 43.4 | 47.3 | 39.4 | 36.8 | 74.8 | 49.0 | | | | | | |

a Not sown.

b Average for 3 years.

**TABLE VII.**—*Germination percentages of seed of Pinus contorta at various storage points*

[Leaders indicate "no germination"]

| Storage point and temperature condition. | 1910, 95-day test. | | | | | | 1911, 77-day test. | | | | | | 1912, 93-day test. | | | | | | 1914, 90-day test. | | | | | | Average for all containers for all 4 years. | Average for each container at all 3 temperatures for all 4 years. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. |
| Ann Arbor, Mich.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Fluctuating* | 71.0 | 92.5 | 58.0 | 15.5 | 87.0 | 64.8 | 42.5 | 62.0 | 21.0 | 14.5 | 69.0 | 41.8 | 33.0 | 39.0 | 38.5 | 31.0 | 70.0 | 42.5 | 2.0 | 14.0 | 0.5 | | 14.5 | 6.2 | 38.8 | | | | | |
| Indoor | 80.0 | 64.0 | 53.5 | 19.0 | 74.5 | 58.6 | 39.5 | 74.5 | 30.5 | .5 | 45.5 | 39.3 | 61.0 | 59.5 | 40.0 | 24.5 | 70.0 | 51.0 | 12.0 | 31.0 | 11.5 | 7.5 | 21.0 | 16.6 | 41.4 | | | | | |
| Low | 65.0 | 76.5 | 8.0 | 1.5 | 72.5 | 44.7 | 40.0 | 23.0 | 23.0 | .5 | 60.5 | 29.4 | 38.0 | 47.5 | 40.0 | 11.5 | 59.0 | 39.2 | 55.0 | 65.5 | 21.5 | 21.0 | 66.5 | 46.0 | 39.8 | | | | | |
| Average | 72.0 | 77.7 | 40.5 | 12.0 | 78.0 | 56.0 | 40.7 | 53.2 | 20.8 | 5.2 | 58.3 | 30.8 | 44.0 | 48.7 | 39.5 | 22.7 | 66.3 | 44.2 | 23.0 | 36.8 | 11.2 | 9.5 | 34.0 | 22.9 | 40.0 | 45.0 | 54.0 | 29.4 | 12.4 | 59.2 |
| Dundee, Ill.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Fluctuating* | 69.5 | 80.5 | 45.0 | 4.5 | 86.5 | 57.2 | 44.5 | 43.5 | 50.5 | 15.5 | 79.0 | 47.8 | 34.0 | 38.0 | 37.0 | 6.5 | 76.5 | 38.4 | 15.5 | 46.0 | 15.5 | | 62.0 | 31.8 | 43.8 | | | | | |
| Indoor | 70.0 | 67.5 | 45.0 | 8.0 | 75.0 | 54.3 | 54.5 | 32.5 | 35.5 | 10.5 | 82.0 | 47.0 | 35.0 | 42.5 | 30.0 | 4.5 | 68.0 | 36.0 | 34.0 | 20.5 | 1.0 | .5 | 61.5 | 23.5 | 40.2 | | | | | |
| Low | 64.5 | 81.5 | 49.0 | 8.0 | 62.5 | 53.1 | 52.0 | 69.0 | 52.0 | 44.5 | 76.5 | 58.8 | 35.0 | 31.5 | 38.5 | 12.5 | 64.5 | 36.4 | 25.0 | 30.0 | 23.5 | | 67.5 | 29.2 | 44.4 | | | | | |
| Average | 70.0 | 76.5 | 46.3 | 6.8 | 74.7 | 54.9 | 50.3 | 55.0 | 48.0 | 23.5 | 79.2 | 51.2 | 34.7 | 37.3 | 35.2 | 7.8 | 69.7 | 36.9 | 31.5 | 32.2 | 13.3 | .2 | 63.7 | 28.2 | 42.8 | 46.0 | 50.2 | 15.7 | 9.6 | 71.8 |
| Fort Bayard, N. Mex.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Fluctuating* | 63.5 | 84.0 | 80.0 | 2.5 | 77.5 | 61.5 | 33.0 | 74.5 | 61.0 | . . . | 78.0 | 49.3 | 47.5 | 33.0 | 59.0 | 41.0 | 54.5 | 47.0 | 54.5 | 65.0 | 56.5 | 53.0 | 74.5 | 60.7 | 53.6 | | | | | |
| Indoor | 79.5 | 92.0 | 70.5 | 35.5 | 67.5 | 69.0 | 83.5 | 58.5 | 72.0 | 68.5 | 84.0 | 73.3 | 59.0 | 58.5 | 48.0 | 22.5 | 62.0 | 50.0 | 57.0 | 60.5 | 38.0 | 32.0 | 53.0 | 48.1 | 60.1 | | | | | |
| Low | 58.5 | 68.0 | 66.0 | 17.5 | 85.5 | 59.1 | 73.5 | 56.5 | 58.5 | 29.0 | 80.0 | 59.5 | (a) | (a) | (a) | (a) | (a) | (a) | 43.5 | 45.5 | 42.0 | 41.0 | 63.0 | 47.0 | b55.2 | | | | | |
| Average | 67.2 | 81.3 | 72.2 | 18.5 | 76.8 | 63.2 | 63.3 | 63.2 | 63.8 | 32.5 | 80.7 | 60.7 | 53.3 | 45.8 | 43.5 | 31.8 | 58.3 | 46.5 | 51.7 | 57.0 | 45.5 | 42.0 | 63.5 | 51.9 | 55.6 | 58.9 | 61.8 | 56.2 | 31.2 | 69.8 |
| Halsey, Nebr.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Fluctuating* | 73.0 | 86.5 | 18.0 | 14.5 | 69.5 | 52.3 | 60.5 | 62.0 | 50.0 | 6.0 | 71.5 | 50.0 | 27.5 | 60.0 | 39.5 | 23.5 | 65.0 | 43.1 | 21.5 | 51.5 | 15.5 | 9.0 | 63.0 | 32.1 | 44.4 | | | | | |
| Indoor | 52.5 | 72.5 | 27.5 | 27.0 | 82.5 | 52.4 | 74.0 | 70.5 | 66.0 | 43.0 | 80.5 | 66.8 | 43.0 | 47.5 | 29.0 | 12.5 | 53.0 | 37.0 | 44.0 | 52.5 | 31.0 | . . . | 63.0 | 38.1 | 48.6 | | | | | |
| Low | 50.0 | 71.5 | 64.0 | 2.0 | 52.5 | 48.0 | 48.5 | 43.0 | 62.5 | 47.0 | 72.0 | 54.6 | 46.0 | 41.5 | 38.5 | 6.5 | 66.0 | 39.7 | 36.5 | 47.5 | 37.5 | 6.0 | 7.0 | 26.9 | 42.3 | | | | | |
| Average | 58.5 | 76.8 | 36.5 | 14.5 | 68.2 | 50.9 | 61.0 | 58.5 | 59.5 | 32.0 | 74.7 | 57.1 | 38.8 | 49.7 | 35.7 | 14.2 | 61.3 | 39.9 | 34.0 | 50.5 | 28.0 | 5.0 | 44.3 | 32.4 | 45.1 | 48.1 | 58.9 | 39.9 | 16.4 | 62.1 |
| Ithaca, N. Y.: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Fluctuating* | 78.0 | 70.5 | 31.0 | . . . . | 81.5 | 52.2 | 63.5 | 58.5 | 54.0 | . . . | 60.0 | 47.2 | 39.0 | 46.0 | 31.5 | .5 | 62.0 | 35.8 | 45.0 | 29.5 | 21.5 | .5 | 57.0 | 32.7 | 42.0 | | | | | |
| Indoor | 74.5 | 81.0 | 14.0 | 1.0 | 85.5 | 51.2 | 50.5 | 47.0 | 58.5 | 49.0 | 82.5 | 57.5 | 43.0 | 57.5 | 38.5 | 15.0 | 60.5 | 42.9 | 40.0 | 46.0 | 17.5 | 4.0 | 29.5 | 27.4 | 44.8 | | | | | |
| Low | 52.5 | 80.0 | 72.5 | 8.0 | 72.5 | 57.1 | 49.0 | 57.5 | 44.5 | 41.5 | 78.5 | 54.2 | 38.0 | 36.0 | 35.5 | 29.0 | 64.0 | 40.5 | (a) | (a) | (a) | (a) | (a) | (a) | b50.6 | | | | | |
| Average | 68.3 | 77.2 | 39.2 | 3.0 | 79.8 | 53.5 | 54.3 | 54.3 | 52.3 | 30.2 | 73.7 | 53.0 | 40.0 | 46.5 | 35.2 | 14.8 | 62.2 | 39.7 | 42.5 | 37.8 | 20.0 | 6.8 | 43.3 | 30.1 | 44.1 | 51.3 | 54.0 | 36.7 | 13.7 | 64.8 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lake Clear Junction, N. Y.** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 56.0 | 84.5 | 57.0 | 25.5 | 79.5 | 60.5 | 62.5 | 75.0 | 39.5 | 17.5 | 81.0 | 55.1 | 45.0 | 50.0 | 34.0 | .5 | 58.0 | 17.5 | 41.0 | 51.5 | 22.5 | | 61.0 | 35.2 | 47.1 | | | | | |
| Indoor | 42.0 | 83.0 | 39.0 | 1.5 | 53.5 | 43.8 | 51.0 | 52.0 | 35.0 | 12.5 | 71.5 | 44.4 | 29.5 | 32.5 | 36.5 | 6.0 | 54.5 | 31.8 | 34.0 | 38.5 | 29.0 | 9.0 | 59.5 | 34.0 | 38.5 | | | | | |
| Low | 82.0 | 77.0 | 43.5 | 5.0 | 64.0 | 54.3 | 56.0 | 69.5 | 64.0 | 12.0 | 58.0 | 51.9 | 42.0 | 53.5 | 50.5 | 39.0 | 75.5 | 52.1 | 48.0 | 63.0 | 51.0 | 36.0 | 65.5 | 52.7 | 52.8 | | | | | |
| Average | 60.0 | 81.5 | 46.5 | 10.7 | 65.7 | 52.9 | 56.5 | 65.5 | 46.2 | 24.0 | 70.2 | 50.5 | 38.8 | 45.3 | 40.3 | 15.2 | 62.7 | 40.5 | 41.0 | 51.0 | 34.2 | 15.0 | 62.0 | 40.6 | 46.1 | 49.1 | 60.8 | 41.8 | 13.7 | 65.2 |
| **Lawrence, Kans.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 73.0 | 72.5 | 74.5 | 45.0 | 71.0 | 67.2 | 54.0 | 55.5 | 29.5 | 24.5 | 72.5 | 47.2 | 44.5 | 36.5 | 5.5 | .5 | 59.5 | 20.3 | 10.5 | 10.5 | 1.5 | . . | 48.5 | 14.2 | 39.5 | | | | | |
| Indoor | 64.5 | 64.5 | 57.5 | 2.5 | 65.5 | 50.9 | 44.0 | 46.0 | 53.5 | 5.0 | 77.0 | 45.1 | 45.5 | 49.5 | 25.0 | 1.0 | 57.0 | 35.6 | 25.0 | 52.5 | 1.0 | 3.0 | 51.5 | 26.6 | 39.6 | | | | | |
| Low | 75.5 | 76.5 | 57.5 | .5 | 77.5 | 57.5 | 43.5 | 36.0 | 38.0 | . . | 77.5 | 39.0 | 20.5 | 23.5 | 18.0 | | 69.5 | 27.5 | 22.5 | 31.5 | 24.0 | | 53.0 | 26.2 | 37.6 | | | | | |
| Average | 71.0 | 71.2 | 63.2 | 16.0 | 71.3 | 58.5 | 47.2 | 45.8 | 40.3 | 9.8 | 75.7 | 43.8 | 38.8 | 36.5 | 16.2 | .3 | 62.0 | 30.8 | 19.3 | 31.5 | 8.8 | 1.0 | 51.0 | 22.3 | 38.9 | 44.1 | 46.2 | 32.1 | 6.8 | 65.0 |
| **New Haven, Conn.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 60.5 | 56.5 | 74.5 | 9.0 | 67.0 | 53.5 | 51.5 | 52.5 | 45.0 | 7.5 | 48.5 | 41.0 | 38.5 | 49.5 | 44.5 | . | 65.0 | 39.5 | 27.5 | 40.0 | 32.0 | 2.0 | 71.5 | 34.6 | 42.2 | | | | | |
| Indoor | 85.0 | 77.5 | 27.0 | 26.0 | 75.5 | 58.2 | 67.5 | 68.5 | 49.5 | 7.5 | 66.5 | 51.9 | 58.0 | 61.5 | 43.0 | 26.0 | 67.0 | 51.1 | 51.5 | 63.0 | 34.0 | . . | 58.5 | 41.4 | 50.7 | | | | | |
| Low | 74.0 | 59.5 | 58.5 | . . . . | 90.5 | 56.5 | 34.0 | 43.5 | 42.0 | 1.0 | 87.0 | 41.5 | 10.5 | 18.0 | 17.5 | 5.0 | 70.0 | 24.2 | . . . . | . | . | . | 54.5 | 10.9 | 33.3 | | | | | |
| Average | 73.2 | 64.5 | 53.3 | 11.7 | 77.7 | 56.1 | 51.0 | 54.8 | 45.5 | 5.3 | 67.3 | 44.8 | 35.7 | 43.0 | 35.0 | 10.3 | 67.3 | 38.3 | 26.3 | 34.3 | 22.0 | .7 | 61.5 | 29.0 | 42.1 | 46.6 | 49.2 | 19.0 | 7.0 | 68.4 |
| **Pikes Peak, Colo.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 71.0 | 88.5 | 72.5 | 27.0 | 76.0 | 67.0 | 69.0 | 69.0 | 71.0 | 28.0 | 82.0 | 63.8 | 58.5 | 62.0 | 54.0 | 41.0 | 59.5 | 55.0 | 58.5 | 64.5 | 66.5 | 43.0 | 60.5 | 58.6 | 61.1 | | | | | |
| Indoor | 83.0 | 76.5 | 66.5 | 6.0 | 66.0 | 59.6 | 84.0 | 64.0 | 66.0 | 9.0 | 89.5 | 62.5 | 62.5 | 60.5 | 53.5 | 54.5 | 62.5 | 58.1 | 62.0 | 63.0 | 66.0 | 66.0 | 67.5 | 63.7 | 61.0 | | | | | |
| Low | 53.0 | 68.0 | 80.0 | 75.5 | 82.0 | 71.7 | 46.5 | 44.5 | 47.0 | 17.0 | 84.5 | 47.9 | 24.5 | 32.5 | 38.5 | 23.5 | 68.0 | 37.4 | 36.0 | 39.5 | 39.0 | 39.5 | 68.0 | 44.4 | 50.4 | | | | | |
| Average | 69.0 | 77.7 | 73.0 | 36.2 | 74.7 | 66.1 | 66.5 | 59.2 | 61.3 | 18.0 | 85.3 | 58.1 | 48.5 | 51.7 | 48.7 | 38.7 | 63.3 | 50.2 | 52.2 | 55.7 | 57.2 | 47.5 | 65.3 | 55.6 | 57.5 | 59.0 | 61.1 | 60.0 | 35.1 | 72.2 |
| **Pocatello, Idaho.** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 56.5 | 67.0 | 69.0 | 46.5 | 64.5 | 60.7 | 68.5 | 76.0 | 76.5 | 1.5 | 80.0 | 60.5 | 53.5 | 60.0 | 37.0 | 28.5 | 60.5 | 47.9 | 55.5 | 63.0 | 60.5 | 54.0 | 68.5 | 60.3 | 57.4 | | | | | |
| Indoor | 64.5 | 88.5 | 59.0 | 72.0 | 79.5 | 72.7 | 71.0 | 35.5 | 56.0 | 24.0 | 75.5 | 52.4 | 33.0 | 35.0 | 34.5 | 17.0 | 67.0 | 45.3 | 21.5 | 38.0 | 10.5 | .5 | 65.0 | 27.1 | 40.4 | | | | | |
| Low | 58.0 | 68.0 | 45.5 | 41.5 | 77.5 | 58.1 | 61.5 | 44.5 | 47.5 | 15.5 | 83.0 | 50.4 | 29.0 | 29.0 | 34.5 | 9.0 | 59.0 | 32.1 | 42.0 | 47.0 | 44.0 | 9.5 | 82.5 | 45.0 | 40.6 | | | | | |
| Average | 59.7 | 74.3 | 57.8 | 53.3 | 73.8 | 65.8 | 67.0 | 52.0 | 60.0 | 13.7 | 79.5 | 54.4 | 38.5 | 48.0 | 35.3 | 24.8 | 62.2 | 41.8 | 39.7 | 49.3 | 38.3 | 21.7 | 72.0 | 44.1 | 53.0 | 51.2 | [illegible] | 47.8 | 28.3 | 71.0 |
| **State College, Pa.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 66.0 | 58.0 | 46.0 | 37.5 | 56.0 | 52.7 | 60.0 | 62.5 | 47.5 | 1.0 | 81.5 | 50.5 | 37.5 | 36.5 | 11.5 | 3.0 | 48.0 | 27.7 | 37.5 | 58.0 | 35.5 | 2.5 | 65.0 | 39.7 | 42.7 | | | | | |
| Indoor | 54.0 | 85.5 | 52.0 | .5 | 70.0 | 52.2 | 77.0 | 63.5 | 46.5 | | 80.0 | 53.4 | 49.0 | 48.0 | 15.0 | 6.0 | 44.5 | 36.5 | 24.5 | 46.0 | 15.0 | 6.0 | 45.5 | 27.4 | 42.4 | | | | | |
| Low | 62.5 | 56.5 | 21.5 | 1.0 | 64.5 | 41.2 | 42.5 | 50.5 | 56.5 | | 81.5 | 46.6 | (a) | (a) | (a) | (a) | (a) | (a) | 17.5 | 32.5 | 23.0 | 4.0 | 75.5 | 30.5 | 19.4 | | | | | |
| Average | 60.8 | 66.7 | 39.5 | 13.0 | 63.5 | 48.7 | 59.8 | 58.8 | 50.2 | .3 | 81.7 | 50.2 | 43.3 | 42.3 | 24.3 | 4.5 | 46.1 | 32.1 | 26.5 | 45.5 | 24.5 | 4.2 | 62.0 | 32.5 | 40.0 | 47.6 | [illegible] | 34.0 | 5.5 | 63.4 |
| **Warsaw, Ky.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 70.5 | 66.0 | 33.0 | . | 81.0 | 50.1 | 42.0 | 47.5 | 9.5 | 8.0 | 60.0 | 35.2 | 12.0 | 31.0 | 13.0 | | 58.5 | 25.7 | 18.0 | 32.0 | 10.5 | | 71.5 | 26.3 | 34.1 | | | | | |
| Indoor | 74.5 | 78.0 | 12.0 | 8.0 | 75.0 | 49.5 | 40.5 | 53.5 | 28.5 | . . | 71.5 | 38.8 | 23.5 | 30.5 | 11.5 | 1.5 | 43.5 | 22.5 | 28.5 | 29.0 | 5.5 | 7.0 | 64.0 | 25.6 | 34.2 | | | | | |
| Low | 72.5 | 72.0 | 41.0 | .5 | 73.0 | 51.4 | 49.0 | 60.0 | 33.0 | 7.0 | 80.0 | 45.3 | 23.5 | 31.0 | 12.5 | 2.0 | 51.5 | 24.7 | 10.5 | 18.0 | 6.5 | | 69.0 | 20.8 | 35.7 | | | | | |
| Average | 72.5 | 72.0 | 28.7 | 2.8 | 75.7 | 50.3 | 43.8 | 53.7 | 23.7 | 5.0 | 73.5 | 39.0 | 20.3 | 30.8 | 13.0 | 1.2 | 50.2 | 24.3 | 19.0 | 26.3 | 7.5 | .3 | 68.2 | 24.1 | 34.7 | 39.4 | 45.7 | 18.2 | 4.3 | 66.9 |
| **Waukegan, Ill.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 49.0 | 54.5 | 57.0 | 9.5 | 78.5 | 49.7 | 63.0 | 76.0 | 55.5 | 12.0 | 80.0 | 64.1 | 30.5 | 40.5 | 33.0 | 17.5 | 49.0 | 16.7 | 42.5 | 47.0 | 10.0 | 2.5 | 59.5 | 32.3 | 41.8 | | | | | |
| Indoor | 63.0 | 73.0 | 23.0 | 13.0 | 67.5 | 48.3 | 59.0 | 66.0 | 63.0 | 41.0 | 79.0 | 60.4 | 37.5 | 40.0 | 23.0 | 7.5 | 50.0 | 31.8 | 40.0 | 37.5 | 16.0 | . | 71.5 | 39.2 | 44.7 | | | | | |
| Low | 63.0 | 63.5 | 32.5 | 3.5 | 75.5 | 47.6 | 48.5 | 47.5 | 34.5 | .5 | 82.0 | 42.6 | 25.0 | 30.5 | 23.5 | 22.0 | 56.5 | 31.4 | 11.5 | 24.5 | 37.0 | 4.5 | 70.0 | 29.5 | 37.9 | | | | | |
| Average | 59.0 | 63.7 | 37.5 | 8.7 | 73.8 | 48.5 | 57.5 | 59.5 | 50.8 | 31.2 | 80.7 | 55.8 | 34.0 | 40.8 | 25.8 | 14.0 | 51.8 | 33.3 | 33.3 | 42.7 | 20.7 | 2.3 | 67.7 | 33.8 | 42.7 | 40.0 | 51.7 | 31.6 | 13.0 | 68.4 |
| **Average for all storage points** | 66.2 | 73.0 | 48.8 | 15.9 | 73.4 | 55.7 | 55.3 | 50.4 | 46.3 | 17.0 | 75.4 | 50.5 | 39.1 | 43.5 | 32.8 | 15.3 | 60.7 | 38.3 | 33.6 | 42.5 | 25.6 | 12.1 | 68.7 | 34.5 | . | | | | | |

[a] Not sown.

[b] Average for 5 years.

TABLE VIII.—*Germination percentages of seed of Pinus monticola at various storage points*

[Leaders indicate "no germination"]

| Storage point and temperature condition. | 1910, 122-day test. Paper bag. | 1910 Paper bag paraffined. | 1910 Cloth bag. | 1910 Cloth bag oiled. | 1910 Bottle air-tight. | 1910 Average for all containers. | 1911, 126-day test. Paper bag. | 1911 Paper bag paraffined. | 1911 Cloth bag. | 1911 Cloth bag oiled. | 1911 Bottle air-tight. | 1911 Average for all containers. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ann Arbor, Mich.: | | | | | | | | | | | | |
| Fluctuating | 29.5 | 36.5 | 37.5 | 14.0 | 49.5 | 33.4 | 16.0 | 12.5 | 17.5 | 2.5 | 15.0 | 12.7 |
| Indoor | 34.0 | 44.0 | 35.5 | 29.5 | 46.0 | 37.8 | 13.5 | 11.5 | 15.0 | 5.0 | 11.5 | 11.3 |
| Low | 31.5 | 37.0 | 21.5 | 3.0 | 43.0 | 27.2 | 17.5 | 13.5 | 3.5 | ... | 10.0 | 8.9 |
| Average | 31.7 | 39.2 | 31.5 | 15.5 | 46.2 | 32.8 | 15.7 | 12.5 | 12.0 | 2.5 | 12.2 | 11.0 |
| Dundee, Ill.: | | | | | | | | | | | | |
| Fluctuating | 31.0 | 22.0 | 11.0 | 5.5 | 55.0 | 24.9 | 41.0 | 55.5 | 1.5 | 1.0 | 68.0 | 33.4 |
| Indoor | 45.5 | 29.0 | 29.0 | 4.0 | 64.0 | 34.3 | 36.0 | 34.5 | 2.5 | .5 | 25.0 | 19.7 |
| Low | 49.0 | 38.0 | 14.5 | 1.5 | 63.5 | 33.3 | 12.0 | 9.0 | 1.0 | .5 | 10.0 | 6.5 |
| Average | 41.8 | 29.7 | 18.2 | 3.7 | 60.8 | 30.8 | 29.7 | 33.0 | 1.7 | .7 | 34.3 | 19.9 |
| Fort Bayard, N. Mex.: | | | | | | | | | | | | |
| Fluctuating | 35.5 | 53.0 | 34.5 | 37.5 | 29.5 | 38.0 | 46.0 | 54.0 | 44.0 | 68.0 | 57.5 | 53.9 |
| Indoor | 33.0 | 55.0 | 46.5 | 54.5 | 48.5 | 47.5 | 71.0 | 65.5 | 44.0 | 73.5 | 50.5 | 60.9 |
| Low | 38.0 | 56.5 | 30.5 | 40.0 | 46.0 | 42.2 | 50.5 | 47.0 | 41.5 | 38.0 | 30.5 | 41.5 |
| Average | 35.5 | 54.8 | 37.2 | 44.0 | 41.3 | 42.6 | 55.8 | 55.5 | 43.2 | 59.8 | 46.2 | 52.1 |
| Halsey, Nebr.: | | | | | | | | | | | | |
| Fluctuating | 28.5 | 27.0 | 21.5 | 29.5 | 44.5 | 30.2 | 47.5 | 54.5 | 11.0 | 3.5 | 63.0 | 35.9 |
| Indoor | 47.5 | 43.0 | 39.5 | 23.0 | 40.0 | 38.6 | 65.5 | 78.5 | 22.5 | 32.5 | 44.5 | 48.7 |
| Low | 31.5 | 32.5 | 17.5 | 38.0 | 44.5 | 32.8 | 21.5 | 58.5 | 15.0 | .5 | 50.0 | 29.1 |
| Average | 35.8 | 34.2 | 26.2 | 30.2 | 43.0 | 33.9 | 44.8 | 63.8 | 16.2 | 12.2 | 52.5 | 37.9 |
| Ithaca, N. Y.: | | | | | | | | | | | | |
| Fluctuating | 40.5 | 37.0 | 42.5 | 13.5 | 40.5 | 34.8 | 37.0 | 12.0 | 4.0 | 1.0 | 15.0 | 13.8 |
| Indoor | 35.0 | 31.0 | 29.5 | 7.5 | 24.5 | 25.5 | 30.5 | 17.5 | 18.0 | 7.0 | 49.0 | 24.4 |
| Low | 29.5 | 20.0 | 41.5 | 22.5 | 50.5 | 32.8 | 59.5 | 64.5 | 38.0 | 2.0 | 75.5 | 47.9 |
| Average | 35.0 | 29.3 | 37.8 | 14.5 | 38.5 | 31.0 | 42.3 | 31.3 | 20.0 | 3.3 | 46.5 | 28.7 |

| Storage point and temperature condition. | 1912, 129-day test. Paper bag. | 1912 Paper bag paraffined. | 1912 Cloth bag. | 1912 Cloth bag oiled. | 1912 Bottle air-tight. | 1912 Average for all containers. | 1914, 126-day test. Paper bag. | 1914 Paper bag paraffined. | 1914 Cloth bag. | 1914 Cloth bag oiled. | 1914 Bottle air-tight. | 1914 Average for all containers. | Average for all containers for all 4 years. | Average for each container at all 3 temperatures for all 4 years. Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ann Arbor, Mich.: | | | | | | | | | | | | | | | | | | |
| Fluctuating | 16.0 | 13.5 | 7.0 | 1.5 | 8.5 | 9.3 | . | 0.5 | . | . | 50.0 | 10.1 | 16.4 | | | | | |
| Indoor | 5.0 | 7.5 | . | ... | 9.5 | 4.4 | 0.5 | 6.0 | . | . | 45.0 | 10.3 | 16.0 | | | | | |
| Low | 5.0 | 3.0 | 8.0 | .5 | 10.5 | 5.4 | 1.0 | 2.5 | . | . | 72.5 | 15.2 | 14.2 | | | | | |
| Average | 8.7 | 8.0 | 5.0 | .7 | 9.5 | 6.4 | .5 | 3.0 | .. | | 55.8 | 11.9 | 15.5 | 14.2 | 15.7 | 12.1 | 4.7 | 30.9 |
| Dundee, Ill.: | | | | | | | | | | | | | | | | | | |
| Fluctuating | 9.0 | 11.0 | 2.0 | ... | 5.5 | 5.5 | . | ... | | . | 13.0 | 2.6 | 16.6 | | | | | |
| Indoor | 13.0 | 10.5 | .5 | . | 7.0 | 6.2 | ... | ... | | | 74.0 | 14.8 | 18.8 | | | | | |
| Low | 8.5 | 10.5 | ... | ... | 6.5 | 5.1 | ... | ... | ... | ... | 53.0 | 10.6 | 13.9 | | | | | |
| Average | 10.2 | 10.7 | .8 | . | 6.3 | 5.6 | ... | ... | ... | ... | 46.7 | 9.3 | 16.4 | 20.4 | 18.4 | 5.2 | 1.1 | 37.0 |
| Fort Bayard, N. Mex.: | | | | | | | | | | | | | | | | | | |
| Fluctuating | 5.5 | 7.5 | 5.0 | 3.5 | 4.5 | 5.2 | 33.0 | 38.5 | 19.5 | 9.0 | 37.5 | 27.5 | 31.2 | | | | | |
| Indoor | 6.0 | 6.5 | 10.5 | 9.0 | 7.0 | 7.8 | 44.0 | 44.5 | 21.0 | 7.5 | 65.5 | 36.5 | 38.2 | | | | | |
| Low | (a) | (a) | (a) | (a) | (a) | (a) | 17.5 | 30.5 | 22.0 | 15.0 | 13.5 | 19.7 | [b]34.5 | | | | | |
| Average | 5.8 | 7.0 | 8.0 | 6.2 | 5.8 | 6.5 | 31.5 | 37.8 | 20.8 | 10.5 | 38.8 | 27.9 | 32.3 | 32.2 | 38.8 | 27.3 | 30.1 | 33.0 |
| Halsey, Nebr.: | | | | | | | | | | | | | | | | | | |
| Fluctuating | 2.0 | 9.0 | 2.0 | ... | 5.0 | 3.6 | ... | 1.5 | ... | ... | 28.5 | 6.0 | 18.9 | | | | | |
| Indoor | 6.5 | 9.0 | 5.5 | . | 10.0 | 6.2 | 1.0 | 9.5 | ... | | 17.5 | 5.6 | 24.8 | | | | | |
| Low | 13.5 | 14.5 | 8.0 | | 6.5 | 8.5 | ... | 1.5 | .. | | 28.5 | 6.0 | 19.1 | | | | | |
| Average | 7.3 | 10.8 | 5.8 | .. | 7.2 | 6.2 | .3 | 4.2 | . | .. | 24.8 | 5.9 | 21.0 | 22.0 | 28.2 | 11.0 | 10.6 | 31.9 |
| Ithaca, N. Y.: | | | | | | | | | | | | | | | | | | |
| Fluctuating | 10.5 | 3.5 | 5.0 | .. | 5.0 | 4.8 | .. | 6.0 | ... | ... | 18.5 | 4.9 | 14.6 | | | | | |
| Indoor | 5.0 | 3.0 | .5 | 1.5 | 9.5 | 3.9 | 2.0 | 7.5 | .5 | .. | 35.0 | 9.0 | 15.7 | | | | | |
| Low | 11.5 | 15.5 | .5 | .5 | 5.5 | 6.7 | (a) | (a) | (a) | (a) | (a) | (a) | [b]29.1 | | | | | |
| Average | 9.0 | 7.3 | 2.0 | .7 | 6.7 | 5.1 | 1.0 | 6.8 | .2 | ... | 26.8 | 7.0 | 18.0 | 21.8 | 18.7 | 15.0 | 4.6 | 19.6 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lake Clear Junction, N. Y.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 47.5 | 46.5 | 44.0 | 29.0 | 24.5 | 38.3 | 76.5 | 83.0 | 36.5 | 3.0 | 9.0 | 41.6 | 4.5 | 6.0 | 2.0 | .... | 8.0 | 4.1 | 15.5 | 16.5 | ... | ... | 27.5 | 11.9 | 24.0 | | | | | |
| Indoor | 29.0 | 35.5 | 33.5 | 23.5 | 19.5 | 28.2 | 13.5 | 8.0 | 20.5 | 1.0 | 5.0 | 9.6 | 9.5 | 9.5 | 6.0 | .... | 11.0 | 7.2 | 21.5 | 27.0 | .5 | ... | 15.0 | 12.8 | 14.5 | | | | | |
| Low | 24.0 | 25.5 | 24.0 | 30.0 | 31.0 | 26.9 | 3.0 | 5.0 | 7.0 | 15.0 | 11.0 | 8.2 | 10.5 | 6.5 | 5.0 | 1.0 | 7.5 | 6.1 | 18.5 | 24.0 | 4.0 | .5 | 20.5 | 13.5 | 13.7 | | | | | |
| Average | 33.5 | 35.8 | 33.8 | 27.5 | 25.0 | 31.1 | 31.0 | 32.0 | 21.3 | 6.3 | 8.3 | 19.8 | 8.2 | 7.3 | 4.3 | .3 | 8.8 | 5.8 | 18.5 | 22.5 | 1.5 | .2 | 21.0 | 12.7 | 17.4 | 22.8 | 24.4 | 15.2 | 8.6 | 15.8 |
| **Lawrence, Kans.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 20.5 | 28.0 | 7.5 | 1.0 | 49.5 | 21.3 | .... | .5 | .... | .... | 10.5 | 2.2 | .5 | .... | .... | 22.5 | 24.0 | 9.4 | .... | .... | .... | .... | 50.5 | 10.2 | 10.8 | | | | | |
| Indoor | 37.5 | 50.0 | 10.0 | .5 | 37.0 | 27.0 | 2.5 | 6.0 | 4.0 | .... | 69.5 | 16.4 | .... | .5 | .... | .... | 12.5 | 2.6 | .... | .... | .... | .... | 34.5 | 6.9 | 13.2 | | | | | |
| Low | 28.0 | 26.5 | 1.5 | .... | 49.5 | 21.1 | 1.5 | 7.5 | .5 | .... | 25.5 | 7.0 | .... | .... | .... | .... | 9.0 | 1.8 | .... | .... | .... | .... | 25.0 | 5.0 | 8.7 | | | | | |
| Average | 28.7 | 34.8 | 6.3 | .5 | 45.3 | 23.1 | 1.3 | 4.7 | 1.5 | .... | 35.2 | 8.5 | .2 | .2 | .... | 7.5 | 15.2 | 4.6 | .... | .... | .... | .... | 36.7 | 7.3 | 10.9 | 7.6 | 9.9 | 2.0 | 2.0 | 33.1 |
| **New Haven, Conn.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 43.0 | 46.5 | 24.0 | 7.0 | 42.0 | 32.5 | 11.0 | 21.5 | .5 | .... | 35.5 | 13.7 | 1.0 | .5 | .... | ... | 6.0 | 1.5 | .... | .... | .... | .... | 16.5 | 3.3 | 12.8 | | | | | |
| Indoor | 35.0 | 37.0 | 35.5 | 27.5 | 49.0 | 36.8 | 50.0 | 26.0 | 6.5 | 1.5 | 5.5 | 17.9 | 4.5 | 3.5 | .... | ... | 5.5 | 2.7 | .... | 2.0 | ... | .... | 15.0 | 3.4 | 15.2 | | | | | |
| Low | 38.5 | 47.0 | 38.5 | 5.0 | 54.0 | 36.6 | 1.5 | .5 | 4.0 | 1.5 | 8.0 | 3.1 | .... | ... | .... | .... | 3.5 | .7 | .... | .... | .... | .... | 47.0 | 9.4 | 12.5 | | | | | |
| Average | 38.8 | 43.5 | 32.7 | 13.2 | 48.3 | 35.3 | 20.8 | 16.0 | 3.7 | 1.0 | 16.3 | 11.6 | 1.8 | 1.3 | .... | .... | 5.0 | 1.6 | .... | .7 | .... | .... | 26.2 | 5.4 | 13.5 | 15.4 | 15.4 | 9.1 | 3.6 | 24.0 |
| **Pikes Peak, Colo.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 27.5 | 38.0 | 37.0 | 39.0 | 36.5 | 35.6 | 5.5 | 6.0 | 7.5 | 19.0 | 6.0 | 8.8 | 4.5 | 1.0 | 7.0 | 13.0 | 7.5 | 6.6 | 14.0 | 14.5 | 21.0 | 22.5 | 10.5 | 16.5 | 16.9 | | | | | |
| Indoor | 34.0 | 36.0 | 43.0 | 50.5 | 41.0 | 40.9 | 8.5 | 5.0 | 6.0 | 25.0 | 6.5 | 10.1 | 4.0 | 11.0 | 7.5 | 16.0 | 9.5 | 9.6 | 10.5 | 28.0 | 34.0 | 39.0 | 25.5 | 27.4 | 22.0 | | | | | |
| Low | 36.5 | 41.0 | 34.0 | 37.0 | 41.5 | 38.0 | 13.0 | 12.0 | 10.5 | 12.5 | 8.0 | 11.2 | 6.5 | 6.0 | 7.5 | 2.0 | 4.0 | 5.2 | ... | .5 | .... | .... | 20.5 | 4.2 | 14.7 | | | | | |
| Average | 32.7 | 38.3 | 38.0 | 42.2 | 39.7 | 38.2 | 9.0 | 7.7 | 8.0 | 18.8 | 6.8 | 10.1 | 5.0 | 6.0 | 7.3 | 10.3 | 7.0 | 7.1 | 8.2 | 14.3 | 18.3 | 20.5 | 18.8 | 16.0 | 17.9 | 13.7 | 16.6 | 17.9 | 23.0 | 18.1 |
| **Pocatello, Idaho:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 40.0 | 30.5 | 39.5 | 24.5 | 36.0 | 34.1 | 7.0 | 5.5 | 10.0 | 32.5 | 11.5 | 13.3 | 2.5 | 4.5 | 8.0 | 16.0 | 6.5 | 7.5 | 16.0 | 19.0 | 16.5 | 7.5 | 19.5 | 15.7 | 17.7 | | | | | |
| Indoor | 33.5 | 41.0 | 41.5 | 30.0 | 43.5 | 37.9 | 12.5 | 21.0 | 15.0 | 20.0 | 9.0 | 15.5 | 18.5 | 11.0 | 18.5 | 20.0 | 6.5 | 14.9 | 1.0 | 3.0 | . | .5 | 33.0 | 7.5 | 19.0 | | | | | |
| Low | 40.0 | 34.5 | 37.0 | 25.0 | 42.0 | 35.7 | 11.0 | 17.5 | 10.0 | 9.5 | 17.5 | 13.1 | 10.5 | 10.0 | 12.0 | 5.5 | 5.0 | 8.6 | 2.0 | 6.0 | .3 | . | 11.5 | 4.0 | 15.4 | | | | | |
| Average | 37.8 | 35.3 | 39.3 | 26.5 | 40.5 | 35.9 | 10.2 | 14.7 | 11.7 | 20.7 | 12.7 | 14.0 | 10.5 | 8.5 | 12.8 | 13.8 | 6.0 | 10.3 | 6.3 | 9.0 | 5.7 | 2.7 | 21.3 | 9.3 | 17.3 | 16.2 | 16.9 | 17.4 | 15.9 | 20.1 |
| **State College, Pa.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 36.0 | 38.5 | 40.5 | 35.0 | 51.0 | 40.2 | 23.5 | 9.0 | 5.5 | 7.5 | 16.0 | 9.3 | 14.5 | 8.5 | 1.5 | 4.0 | 5.0 | 6.7 | .... | .5 | . | .. | 15.0 | 3.1 | 14.8 | | | | | |
| Indoor | 43.0 | 22.0 | 35.5 | 13.0 | 35.0 | 20.7 | 23.0 | 20.0 | 33.5 | 3.5 | 18.0 | 19.6 | 13.0 | 11.0 | .. | ... | 11.5 | 7.1 | .... | .5 | . | .. | 37.5 | 7.6 | 16.0 | | | | | |
| Low | 25.5 | 18.5 | 33.5 | 31.5 | 49.5 | 31.7 | 25.0 | 26.5 | 5.0 | .5 | 8.5 | 15.1 | (a) | (a) | (a) | (a) | (a) | (a) | .... | ... | . | ... | 11.5 | 2.3 | [b]15.7 | | | | | |
| Average | 34.8 | 26.3 | 36.5 | 26.5 | 45.2 | 33.9 | 20.5 | 18.5 | 14.7 | 2.2 | 14.2 | 14.0 | 11.8 | 9.8 | .8 | 2.0 | 8.2 | 6.9 | ... | .3 | . | ... | 21.3 | 4.3 | 14.8 | 17.3 | 13.7 | 13.0 | 7.7 | 22.2 |
| **Warsaw, Ky.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 46.5 | 41.5 | 22.5 | .5 | 44.0 | 31.0 | 4.0 | 13.0 | .. | .. | 8.5 | 5.1 | . | ... | . | .... | 6.5 | 1.3 | ... | ... | . | ... | 24.0 | 4.8 | 10.6 | | | | | |
| Indoor | 33.0 | 45.0 | 17.5 | 2.0 | 30.0 | 27.3 | 1.0 | 9.0 | . | .. | 12.5 | 4.5 | ... | ... | . | ... | 9.5 | 1.9 | ... | ... | . | .. | 21.5 | 4.3 | 9.5 | | | | | |
| Low | 34.0 | 31.5 | 19.0 | 0.5 | 31.5 | 24.5 | 0.0 | 13.0 | | ... | 13.0 | 6.4 | .. | . | . | ... | 15.5 | 3.1 | .. | .. | . | .... | 25.5 | 5.1 | 9.8 | | | | | |
| Average | 37.8 | 39.3 | 19.7 | 3.0 | 38.2 | 27.0 | 3.7 | 11.7 | . | ... | 11.3 | 5.3 | . | .. | ... | ... | 10.5 | 2.1 | | . | ... | .. | 23.7 | 4.7 | 10.0 | 10.4 | 12.8 | 4.9 | .8 | 20.9 |
| **Waukegan, Ill.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 41.5 | 32.0 | 32.0 | 6.5 | 32.0 | 28.8 | 16.0 | 24.5 | 9.5 | 4.0 | 44.5 | 19.3 | 9.5 | 8.5 | | . | 9.0 | 5.4 | . | . | ... | ... | 25.0 | 5.0 | 14.6 | | | | | |
| Indoor | 31.0 | 40.5 | 21.0 | 20.0 | 28.5 | 28.2 | 15.0 | 11.5 | 5.5 | 4.5 | 12.5 | 10.4 | 6.5 | 11.5 | .. | ... | 10.5 | 5.7 | .. | 1.0 | . | .. | 22.0 | 4.6 | 12.2 | | | | | |
| Low | 30.5 | 42.5 | 33.5 | 11.5 | 36.0 | 30.8 | 10.5 | 14.0 | 3.0 | . | 29.5 | 11.4 | 5 | 2.0 | ... | .... | 11.0 | 2.7 | . | . | ... | ... | 16.0 | 3.2 | 12.0 | | | | | |
| Average | 34.3 | 38.3 | 28.8 | 12.7 | 32.2 | 29.3 | 13.8 | 16.7 | 7.0 | 2.2 | 28.8 | 13.7 | 5.5 | 7.3 | .... | | 10.2 | 4.6 | .. | .3 | .. | ... | 21.0 | 4.3 | 12.9 | 15.4 | 15.6 | 9.0 | 3.7 | 23.0 |
| **Average all storage points** | 35.2 | 36.8 | 29.7 | 20.0 | 45.9 | 32.7 | 23.0 | 24.5 | 12.4 | 10.0 | 25.0 | 19.0 | 6.6 | 6.5 | 3.6 | 3.7 | 8.2 | 5.6 | 5.1 | 7.6 | 3.6 | 2.6 | 29.5 | 9.7 | ... | | | | | |

a Not sown.

b Average for 3 years.

TABLE IX.—*Germination percentages of seed of Pinus strobus at various storage points*

[Leaders indicate "no germination"]

| Storage point and temperature condition. | 1910, 115-day test. | | | | | | 1911, 126-day test. | | | | | | 1912, 129-day test. | | | | | | 1914, 126-day test | | | | | | Average for all containers for 4 years. | Average for each container at all temperatures for all 4 years. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. | Average for all containers. | Paper bag. | Paper bag paraffined. | Cloth bag | Cloth bag oiled | Bottle air-tight. | Average for all containers. | | Paper bag. | Paper bag paraffined. | Cloth bag. | Cloth bag oiled. | Bottle air-tight. |
| **Ann Arbor, Mich.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 28.0 | 30.5 | 18.5 | 31.0 | 48.5 | 31.3 | 39.0 | 30.0 | 5.5 | ... | 42.0 | 23.3 | 8.5 | 15.5 | 14.0 | ... | 31.0 | 13.8 | ... | ... | | ... | 64.0 | 12.8 | 20.4 | | | | | |
| Indoor | 53.0 | 49.0 | 44.5 | 46.0 | 53.5 | 49.2 | 61.0 | 40.0 | 5.0 | 0.5 | 47.5 | 30.8 | | 14.5 | ... | ... | 31.0 | 9.1 | ... | | ... | ... | 60.0 | 12.0 | 25.3 | | | | | |
| Low | 38.5 | 35.5 | 12.5 | 1.0 | 56.0 | 28.7 | 36.0 | 38.5 | 1.0 | ... | 49.0 | 24.9 | 5.5 | 9.5 | | ... | 27.5 | 8.5 | ... | | ... | ... | 28.5 | 5.7 | 17.0 | | | | | |
| Average | 39.8 | 38.3 | 25.2 | 26.0 | 52.7 | 36.4 | 45.3 | 36.2 | 3.8 | .2 | 46.2 | 26.3 | 4.7 | 13.2 | 4.7 | ... | 29.8 | 10.5 | ... | ... | | ... | 50.8 | 10.2 | 20.9 | 22.4 | 21.9 | 8.4 | 6.6 | 44.9 |
| **Dundee, Ill.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 32.0 | 40.5 | 23.0 | 12.5 | 53.5 | 32.3 | 20.0 | 27.5 | 1.5 | ... | 40.0 | 17.8 | .3 | 2.5 | ... | ... | 33.0 | 7.2 | ... | | | | 51.5 | 10.3 | 16.9 | | | | | |
| Indoor | 31.5 | 33.5 | 28.0 | ... | 58.5 | 30.3 | 34.0 | 39.0 | 1.5 | | 44.0 | 23.7 | .5 | 11.0 | ... | ... | 33.5 | 9.0 | | ... | | | 56.5 | 11.3 | 18.6 | | | | | |
| Low | 28.0 | 30.5 | 18.5 | ... | 50.0 | 25.4 | 25.0 | 38.5 | .5 | | 55.0 | 23.8 | 2.5 | 18.0 | ... | ... | 22.0 | 8.5 | ... | ... | | ... | 56.0 | 11.2 | 17.2 | | | | | |
| Average | 30.5 | 34.8 | 23.2 | 4.2 | 54.0 | 29.3 | 26.3 | 35.0 | 1.2 | ... | 46.3 | 21.8 | 1.2 | 10.5 | | | 29.5 | 8.2 | ... | | | | 54.7 | 10.9 | 17.6 | 14.5 | 20.1 | 6.1 | 1.0 | 46.1 |
| **Fort Bayard, N. Mex.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 33.0 | 37.5 | 54.5 | 60.5 | 58.5 | 48.8 | 40.5 | 40.0 | 36.0 | 31.0 | 56.5 | 40.8 | 21.0 | 18.5 | 31.5 | 36.5 | 38.0 | 29.1 | 28.5 | 27.0 | 23.0 | 8.5 | 60.5 | 29.5 | 37.1 | | | | | |
| Indoor | 54.0 | 54.0 | 50.5 | 51.0 | 47.0 | 51.3 | 41.5 | 47.0 | 39.5 | 42.0 | 49.5 | 43.9 | 33.5 | 37.5 | 29.0 | 16.0 | 38.0 | 29.6 | 3.0 | 2.5 | ... | ... | .5 | 1.2 | 31.5 | | | | | |
| Low | 60.0 | 47.5 | 73.5 | 42.5 | 53.5 | 55.4 | 51.5 | 51.0 | 47.0 | 42.5 | 58.0 | 50.0 | (a) | (a) | (a) | (a) | (a) | (a) | 19.0 | 30.0 | 19.0 | 9.0 | (a) | 19.3 | [b]43.1 | | | | | |
| Average | 49.0 | 46.3 | 59.5 | 51.3 | 53.0 | 51.8 | 44.5 | 46.0 | 40.8 | 38.5 | 54.7 | 44.9 | 27.2 | 25.0 | 30.2 | 26.2 | 38.0 | 29.3 | 16.8 | 19.8 | 14.0 | 5.8 | 30.5 | 16.7 | 35.7 | 34.4 | 34.3 | 36.1 | 30.4 | 44.0 |
| **Halsey, Nebr.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 46.5 | 43.0 | 29.5 | 36.0 | 49.5 | 40.9 | 26.5 | 40.5 | 9.0 | ... | 52.5 | 25.7 | ... | 3.0 | ... | ... | 26.5 | 5.9 | ... | ... | | | 58.5 | 11.7 | 21.1 | | | | | |
| Indoor | 34.5 | 42.0 | 42.0 | 3.0 | 61.5 | 30.6 | 37.0 | 48.0 | ... | 4.5 | 58.0 | 29.5 | 18.0 | 9.0 | ... | | 39.0 | 13.2 | ... | ... | ... | ... | 55.5 | 11.1 | 22.6 | | | | | |
| Low | 41.5 | 52.0 | 21.0 | 49.5 | 57.5 | 43.3 | 21.5 | 29.5 | | | 47.0 | 19.6 | 13.0 | 14.5 | 2.5 | ... | 26.5 | 11.3 | ... | ... | | | 37.5 | 7.5 | 20.4 | | | | | |
| Average | 40.8 | 45.7 | 30.8 | 29.5 | 54.5 | 40.3 | 28.3 | 39.3 | 3.0 | 1.5 | 52.5 | 24.9 | 10.3 | 8.8 | .8 | | 30.7 | 10.1 | ... | ... | ... | ... | 50.5 | 10.1 | 21.4 | 19.8 | 23.4 | 8.6 | 7.8 | 47.0 |
| **Ithaca, N. Y.:** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fluctuating | 44.5 | 47.5 | 10.0 | 35.0 | 55.5 | 38.5 | 26.5 | 37.0 | 23.5 | ... | 54.5 | 28.3 | 1.0 | 6.5 | ... | ... | 23.5 | 6.2 | ... | | | ... | 47.5 | 9.5 | 20.6 | | | | | |
| Indoor | 56.5 | 54.5 | 42.0 | 15.0 | 50.5 | 43.7 | 29.5 | 43.5 | 4.0 | 1.5 | 55.5 | 26.8 | 9.0 | 14.5 | .5 | ... | 36.5 | 12.1 | ... | ... | ... | ... | 55.0 | 11.0 | 23.4 | | | | | |
| Low | 36.5 | 39.5 | 31.0 | 8.5 | 60.5 | 35.2 | 32.0 | 33.5 | 15.0 | 2.0 | 45.5 | 25.6 | ... | 13.0 | ... | ... | 26.5 | 7.9 | (a) | (a) | (a) | (a) | (a) | (a) | [b]22.9 | | | | | |
| Average | 45.8 | 47.2 | 27.7 | 19.5 | 55.5 | 39.1 | 29.3 | 38.0 | 14.2 | 1.2 | 51.8 | 26.9 | 3.3 | 11.3 | .2 | ... | 28.8 | 8.7 | ... | ... | ... | ... | 51.2 | 10.2 | 21.2 | 19.6 | 24.1 | 10.5 | 5.2 | 46.8 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lake Clear Junction, N. Y.:** | | | | | | | | | | | | | | | |
| Fluctuating | 55.5 | 60.0 | 55.0 | 11.5 | 57.0 | 47.8 | 41.5 | 43.5 | 45.5 | .5 | 41.5 | 30.5 | 20.5 | 15.5 | 1.5 |
| Indoor | 55.5 | 60.0 | 39.5 | 34.0 | 66.5 | 51.1 | 27.0 | 38.0 | 35.0 | 43.0 | 49.5 | 38.5 | 23.5 | 16.0 | |
| Low | 40.5 | 54.5 | 50.5 | 28.0 | 66.0 | 47.9 | 41.5 | 44.0 | 30.5 | 20.5 | 52.5 | 39.0 | 23.0 | 20.0 | 1.5 |
| Average | 50.5 | 58.2 | 48.3 | 24.5 | 63.2 | 48.9 | 36.7 | 41.8 | 37.5 | 21.3 | 47.8 | 36.0 | 21.7 | 17.2 | 1.0 |
| **Lawrence, Kans.:** | | | | | | | | | | | | | | | |
| Fluctuating | .3 | 44.5 | ... | .. | 54.0 | 19.8 | . | .5 | | .. | 48.0 | 9.7 | . | | .. |
| Indoor | 27.0 | 35.0 | ... | . | 62.0 | 24.8 | 1.0 | 3.0 | ... | .. | 50.0 | 10.8 | | . | . |
| Low | 10.0 | 24.0 | .5 | .... | 52.5 | 17.4 | | 1.0 | | . | 46.5 | 9.5 | | . | .. |
| Average | 12.5 | 34.5 | .2 | ... | 56.2 | 20.7 | .3 | 1.5 | | | 48.2 | 10.0 | | . | . |
| **New Haven, Conn.:** | | | | | | | | | | | | | | | |
| Fluctuating | 32.5 | 33.0 | 11.5 | 4.5 | 61.0 | 28.5 | 10.5 | 13.5 | .5 | | 43.5 | 13.6 | . | . | . |
| Indoor | 48.0 | 50.0 | 44.5 | 20.0 | 56.0 | 41.7 | 32.0 | 31.5 | 2.0 | | 49.0 | 22.9 | 4.5 | 12.0 | .. |
| Low | 31.0 | 32.5 | 29.5 | 23.5 | 60.5 | 35.4 | . | . | .5 | . | 49.0 | 9.9 | | | .. |
| Average | 37.2 | 38.5 | 28.5 | 12.7 | 59.2 | 35.2 | 14.2 | 15.0 | 1.0 | | 47.2 | 15.5 | 1.5 | 4.0 | .. |
| **Pikes Peak, Colo.:** | | | | | | | | | | | | | | | |
| Fluctuating | 36.5 | 56.0 | 56.5 | 57.0 | 53.5 | 55.9 | 55.5 | 50.5 | 51.0 | 45.5 | 48.0 | 50.1 | 19.5 | 17.5 | 31.0 |
| Indoor | 59.5 | 62.5 | [illegible] | 58.5 | 62.0 | 59.1 | 40.0 | 30.5 | 39.5 | 31.0 | 50.5 | 38.3 | 16.5 | 23.0 | 33.0 |
| Low | 26.0 | 33.5 | 39.0 | 18.5 | 57.0 | 34.8 | 13.5 | 15.5 | 5.0 | 5.0 | 44.0 | 16.6 | . | 3.0 | 3.0 |
| Average | 47.3 | 50.7 | 49.5 | 44.7 | 57.5 | 49.9 | 36.3 | 32.2 | 31.8 | 27.2 | 47.5 | 35.0 | 12.0 | 14.5 | 22.3 |
| **Pocatello, Idaho:** | | | | | | | | | | | | | | | |
| Fluctuating | 68.5 | 57.0 | 61.5 | 63.0 | 58.0 | 61.6 | 36.0 | 42.0 | 54.0 | 26.0 | 36.5 | 38.9 | 21.0 | 21.0 | 40.5 |
| Indoor | 60.0 | 61.5 | 63.5 | 59.0 | 63.5 | 61.5 | 42.0 | 37.5 | 42.5 | 22.0 | 77.0 | 44.2 | 18.5 | 23.0 | 12.[illegible] |
| Low | 37.5 | 41.0 | 41.0 | 21.5 | 58.5 | 39.9 | 45.0 | 46.5 | 33.0 | 17.5 | 49.5 | 38.3 | 12.0 | 21.0 | 6.0 |
| Average | 55.3 | 53.2 | 55.3 | 47.8 | 60.0 | 54.3 | 41.0 | 42.0 | 43.2 | 21.8 | 54.3 | 40.5 | 17.2 | 21.7 | 16.3 |
| **State College, Pa.:** | | | | | | | | | | | | | | | |
| Fluctuating | 16.5 | 41.5 | 38.0 | 3.5 | 50.5 | 34.0 | 30.5 | 26.5 | 13.0 | | 45.0 | 27.0 | [illegible] | [illegible] | . |
| Indoor | 50.0 | 52.0 | 35.5 | 21.5 | 56.5 | 43.1 | 25.5 | 29.0 | 3.0 | 1.0 | 39.0 | 19.9 | [illegible] | 1.0 | |
| Low | 28.0 | 32.5 | 34.5 | 9.0 | 64.0 | 33.6 | 32.5 | 27.5 | 17.0 | | 41.0 | 23.0 | (a) | (a) | (a) |
| Average | 18.2 | 42.0 | 36.0 | 11.3 | 57.0 | 36.9 | 29.5 | 27.7 | 18.3 | .3 | 41.7 | 23.5 | 8.8 | 9.8 | |
| **Warsaw, Ky.:** | | | | | | | | | | | | | | | |
| Fluctuating | 36.0 | 35.5 | 1.0 | . | 55.5 | 25.6 | | 7.5 | | | 45.5 | 10.6 | | | . |
| Indoor | 25.0 | 41.0 | . | . | 56.0 | 24.4 | 2.5 | 8.5 | . | | 33.0 | 8.8 | . | | . |
| Low | .5 | 34.0 | 1.0 | . | 66.0 | 20.3 | 4.0 | 13.0 | | . | 34.0 | 10.7 | . | | |
| Average | 20.5 | 36.8 | .7 | . | 59.2 | 23.4 | 2.2 | 9.7 | . | | 37.5 | 9.9 | | | |
| **Waukegan, Ill.:** | | | | | | | | | | | | | | | |
| Fluctuating | 57.5 | 46.0 | 37.5 | 4.0 | 75.0 | 41.6 | 10.5 | 30.0 | | | 51.0 | 12.1 | 5.5 | 10.0 | |
| Indoor | 41.5 | 53.5 | 12.0 | 3.0 | 67.0 | 35.4 | .5 | 32.5 | | | 55.5 | 17.7 | | 11.5 | |
| Low | 33.5 | 34.0 | 2.0 | | 69.0 | 27.7 | | 21.5 | 3.5 | .5 | 64.5 | 18.6 | .. | .5 | |
| Average | 44.2 | 44.5 | 17.2 | 2.3 | 60.7 | 35.0 | 10.3 | 29.0 | 1.2 | .2 | 55.0 | 19.5 | 1.8 | 7.3 | |
| **Average all storage points** | 39.4 | 43.9 | 36.9 | 21.1 | 57.8 | 38.6 | 26.5 | 30.3 | 14.7 | 8.6 | 48.7 | 25.7 | 8.4 | 11.0 | 5.8 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lake Clear Junction, N. Y.:** | | | | | | | | | | | | | | | |
| Fluctuating | | 26.0 | 12.7 | 7.5 | 9.0 | | | 11.0 | 9.5 | 25.[illegible] | | | | | |
| Indoor | | 24.0 | 12.7 | 6.5 | 13.0 | | . | 37.0 | 11.3 | 28.4 | | | | | |
| Low | | 33.5 | 15.2 | 35.0 | 19.0 | 7.0 | . | 47.5 | 21.7 | 31.0 | | | | | |
| Average | | 27.8 | 13.5 | 16.3 | 13.7 | 2.3 | . | 38.5 | 14.2 | 18.2 | 11.5 | 33.7 | 21.0 | 11.4 | 44.4 |
| **Lawrence, Kans.:** | | | | | | | | | | | | | | | |
| Fluctuating | .5 | 45.0 | 9.1 | ... | ... | . | | 32.0 | 6.4 | 11.3 | | | | | |
| Indoor | . | 44.5 | 8.9 | .... | | . | . | 61.0 | 12.2 | 14.2 | | | | | |
| Low | . | 28.5 | 5.7 | . | | . | | 72.5 | 14.5 | 11.8 | | | | | |
| Average | .2 | 39.3 | 7.9 | . | . | . | | 55.2 | 11.0 | 12.4 | 3.7 | 0.0 | .05 | .05 | 49.7 |
| **New Haven, Conn.:** | | | | | | | | | | | | | | | |
| Fluctuating | . | 31.5 | 6.4 | ... | | .. | | 44.0 | 8.8 | 14.3 | | | | | |
| Indoor | ... | 33.5 | 10.0 | . | . | .. | | 60.0 | 12.0 | 21.7 | | | | | |
| Low | | 21.0 | 4.2 | | | | | 14.5 | 2.9 | 13.1 | | | | | |
| Average | | 28.7 | 6.8 | | | | | 30.5 | 7.9 | 16.4 | 13.2 | 14.3 | 7.4 | 3.2 | 43.6 |
| **Pikes Peak, Colo.:** | | | | | | | | | | | | | | | |
| Fluctuating | 17.0 | 33.0 | 25.0 | 52.0 | 41.0 | 47.5 | 19.5 | 43.0 | 40.6 | 42.6 | | | | | |
| Indoor | 41.0 | 32.0 | 29.5 | 48.0 | 37.0 | 42.0 | 22.5 | 54.0 | 40.7 | 41.9 | | | | | |
| Low | | 27.0 | 6.6 | | | . | | 53.0 | 30.0 | 17.2 | | | | | |
| Average | 20.0 | 30.7 | 19.0 | 33.3 | 26.0 | 29.8 | 14.0 | 50.0 | 30.6 | 33.0 | 32.2 | [illegible] | 33.4 | 20.5 | 46.4 |
| **Pocatello, Idaho:** | | | | | | | | | | | | | | | |
| Fluctuating | 11.0 | 27.0 | 22.1 | 39.0 | 30.0 | 41.0 | 1.0 | 34.0 | 34.8 | 39.4 | | | | | |
| Indoor | 7.5 | 35.5 | 19.4 | | | | | 64.0 | 13.8 | 34.5 | | | | | |
| Low | 8.0 | 30.5 | 14.5 | 6.0 | 6.0 | . | | 40.5 | 12.1 | 26.1 | | | | | |
| Average | 8.8 | 31.0 | 19.0 | 15.0 | 15.0 | 13.7 | .3 | 55.8 | 20.6 | 33.[illegible] | 32.1 | 33.0 | [illegible] | [illegible] | 59.3 |
| **State College, Pa.:** | | | | | | | | | | | | | | | |
| Fluctuating | | 22.0 | 11.5 | | | | | 19.0 | 9.8 | 20.6 | | | | | |
| Indoor | | 34.5 | 7.2 | | | | | 49.5 | 9.9 | 20.5 | | | | | |
| Low | (a) | (a) | (a) | | | . | | 42.5 | 8.5 | 21.9 | | | | | |
| Average | | 28.2 | 9.4 | | . | | | 47.0 | 9.1 | 19.8 | 19.1 | 19.9 | 14.6 | [illegible] | [illegible] |
| **Warsaw, Ky.:** | | | | | | | | | | | | | | | |
| Fluctuating | | 31.0 | 6.2 | | | | | 51.[illegible] | 10.3 | 11.2 | | | | | |
| Indoor | | 24.0 | 4.8 | | | | | 59.0 | 11.8 | 22.[illegible] | | | | | |
| Low | | 29.5 | 5.9 | | | | | 60.5 | 11.1 | 17.3 | | | | | |
| Average | | 28.2 | 5.6 | | | | | 57.0 | 11.4 | [illegible] | [illegible] | [illegible] | | | [illegible] |
| **Waukegan, Ill.:** | | | | | | | | | | | | | | | |
| Fluctuating | | 11.0 | 0.3 | | | | | 53.5 | 10.7 | 28.5 | | | | | |
| Indoor | | 31.0 | 8.2 | | | | | 47.5 | 9.5 | 17.8 | | | | | |
| Low | | 24.0 | 4.9 | | | | | 46.0 | 9.5 | 15.1 | | | | | |
| Average | | 28.7 | 7.6 | . | | | | 49.0 | 9.8 | 15.1 | 14.1 | [illegible] | 4.6 | | [illegible] |
| **Average all storage points** | 4.2 | 40.7 | 12.0 | 6.3 | [illegible] | 4.6 | 1.5 | 48.4 | 13.[illegible] | | | | | | |

a Not come.

b Average for 3 years.

Various experiments with tree seed go to prove that storage at a uniformly low temperature (0° to 32° F.) is preferable to that at higher temperatures. It is easy to understand that at such low temperatures there would be little if any physiological activity of the seed and consequently little wasting of stored-up food and energy. Had the seed in this study been stored at such low temperatures it is very probable that it would have excelled in germination that stored at the other two temperatures. In this study, however, the seed was stored in basement or cellar where the summer temperature was doubtless fairly high. Moreover, as is usually the case in such locations, the atmospheric humidity was also doubtless high. The conditions, in fact, during summer were such as to be more conducive to physiological activity than under the fluctuating or indoor conditions of storage. Hence, it is reasonabe to suppose that there was more of such activity with the consequent deterioration of the seed. The lesson to be learned from this particular phase of the experiment is to avoid ordinary basements and cellars for storing seed in unsealed containers, and that in northern temperate climates storage indoors where the temperature never goes below freezing is preferable to storage where the temperature follows the natural variations.

## EFFECT OF GEOGRAPHICAL LOCATION

Ordinarily, the seed dealer will perhaps have to store any seed which he possesses at his own establishment regardless of its location. It will be a matter of interest, however, to learn that some geographic locations seem much more suited for the purpose than others. For seed in containers other than the sealed bottle, there was a very consistent superiority shown in the germination of that stored in some locations and a very consistent inferiority of that stored in others. Table III brings this out. Note, for instance, the consistently high relative germination of seed stored at Fort Bayard, Pikes Peak, Pocatello, and Lake Clear Junction; on the other hand, note the relatively low germination of seed stored at Dundee, Lawrence, New Haven, Warsaw, and Waukegan. These are averages for all containers. Knowing, now, that seed in air-tight bottles is not so much affected by adverse conditions as that in other containers, it is believed, accordingly, that it should not be considered in determining the effect of the geographic location upon stored seed. The effect is better shown by the behavior of seed stored in the cloth bags. Using the averages shown in Tables IV to IX, it is found that the towns range themselves in the following order:

| Town. | Average germination percentage for all 4 years of seed stored in cloth bags. | Town. | Average germination percentage for all 4 years of seed stored in cloth bags |
|---|---|---|---|
| Fort Bayard | 44. 1 | State College | 23. |
| Pikes Peak | 39. 0 | Waukegan | 20. |
| Pocatello | 36. 6 | New Haven | 18. |
| Lake Clear Junction | 33. 2 | Dundee | 16. |
| Ithaca | 25. 8 | Lawrence | 13. |
| Halsey | 25. 3 | Warsaw | 9. |
| Ann Arbor | 23. 5 | | |

Fort Bayard stands out as an exceptionally favorable storage point, and Pikes Peak, Pocatello, and Lake Clear Junction follow it rather closely. On the other hand, New Haven, Dundee, Lawrence, Warsaw, and Waukegan seem especially unsuited as locations for seed storage in ordinary containers. There was not enough information collected in connection with this study to show the reason why some points give so much more favorable results than others. It seems probable that it is due to some climatic factor or factors which in turn have their effect upon the physiological activities of the seed. Fort Bayard, Pikes Peak, Pocatello, and Lake Clear Junction are in high altitudes, and the first three places at least experience conditions of low relative atmospheric humidity. Waukegan, Dundee, Lawrence, and Warsaw are middle western locations of moderate altitude which experience severe temperature fluctuations and higher relative humidity than the first three locations mentioned. During the summer when such changes would most affect seed in storage, New Haven experiences higher relative humidities than the middle western locations and severe climatic fluctuations are somewhat less marked there. Regardless of the reason, the study indicates that middle western points and perhaps Atlantic coast points should if possible be avoided as locations for storing coniferous tree seed in ordinary containers, especially for periods of several years. This conclusion for Atlantic coast points may not be entirely warranted as it is based on results at only one station.

There is, however, comfort for those wishing to store seed in such locations when the germination of bottle-stored seed is examined. Based on the average germination percentage of such seed for all species and all years, the towns line up in the following sequence:

| Town. | Average germination percentage for all 4 years of seed stored in air-tight bottles. | Town. | Average germination percentage for all 4 years of seed stored in air-tight bottles. |
|---|---|---|---|
| Dundee | 59.1 | Lawrence | 53.7 |
| Fort Bayard | 57.5 | Lake Clear Junction | 53.1 |
| Waukegan | 56.0 | Ann Arbor | 53.0 |
| Pikes Peak | 55.6 | New Haven | 52.6 |
| Ithaca | 54.8 | State College | 52.3 |
| Pocatello | 54.4 | Warsaw | 52.2 |
| Halsey | 54.4 | | |

This tabulation indicates, and it can be corroborated by inspection of Tables IV to IX, that the bottle-stored seed in this study was not affected by climatic conditions at the points of storage. Two of the points, Dundee and Waukegan, which the study indicates were very unfavorable storage points when ordinary methods of storage are followed, appear in the case of bottle-stored seed to be among the most favorable locations. Here again the lesson is obviously to use air-tight methods of seed storage. The geographic location of the storage point will then be of little or no consequence.

RESULTS OF STORAGE AT END OF TEN YEARS

Reference was made (p. 483) to the fact that some of the bottle-stored seed was carried over for another 5 years and then tested again. The seed so carried over was that of each of the six species which had previously been stored at the indoor temperature at Dundee, Ill. Following the test during the winter of 1914, the bottles were resealed air-tight and then stored until January, 1919, on a shelf in the Forest Service office building at Washington, D. C. The 1914 test of these seed showed a germination percentage of 71.5 for Engelmann spruce, 43 for Douglas fir, 61.5 for lodgepole pine, 82 for western yellow pine, 74 for western white pine, and 56.5 for white pine.

The test in 1919 was carried on for 167 days under the same conditions as in previous years. Engelmann spruce, Douglas fir, and white pine germinated not at all, while lodgepole pine germinated to the extent of 9 per cent, western yellow pine 22 per cent, and western white pine 6.5 per cent. Although the test was continued for 167 days, the lodgepole pine had completed its germination in 90 days, western yellow pine in 75 days, and western white pine in 130 days. Due to the fact that these seeds were exposed to the air at the end of 5 years, although the bottles were afterwards resealed, this particular part of the experiment does not truly indicate whether seed can be successfully stored for 10 years without great deterioration. It does give an idea of the relative sustained vitality of the species concerned. It has been rather apparent all through the study that as respects general sustained vitality, the species will rank in the following sequence, the more vital species being placed first: Western yellow pine, lodgepole pine, western white pine, white pine, Engelmann spruce, and Douglas fir. If the seeds were secured from different sources than those of this study, the sequence might be altered.

# SUSCEPTIBILITY OF THE DIFFERENT VARIETIES OF SWEET POTATOES TO DECAY BY RHIZOPUS NIGRICANS AND RHIZOPUS TRITICI

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## INTRODUCTION

Although *Rhizopus nigricans* Ehrb. has generally been regarded as the cause of the softrot of the sweet potato (*Ipomoea batatas* Lam.), Harter, Weimer, and Lauritzen[1] have shown by recent experiments that a similar decay may be caused by the following additional species of the genus: *tritici* Saito, *nodosus* Namysl, *reflexus* Bainier, *delemar* (Boid), Wehmer and Hanzawa, *oryzae*, Went and Pr. Geerligs, *maydis* Bruderl., *arrhizus* Fischer, and *artocarpi* Racib. Their investigations showed that in order to obtain infection with the different species it was necessary to incubate the potatoes at a temperature suited to the growth of the particular species of Rhizopus with which they were inoculated. They group the different species into high, intermediate, and low temperature forms, *R. nigricans* Ehrb. and *R. tritici*, the two species concerned in the present investigations, belonging to the low and intermediate forms, respectively.

The parasitism of the different species of Rhizopus was determined by them for the Yellow Jersey variety of sweet potatoes only. Although softrot occurs on all the varieties, it is the general belief that some of them are more resistant to decay than others. The Jersey type of sweet potatoes, for example, is recognized as a poor keeper, while some of the varieties grown in the South are thought to keep well in storage. The present investigations were conducted in order to throw more light on the relative susceptibility of a number of the commercial varieties to infection and decay by *R. nigricans* and *R. tritici*.

*R. nigricans* was employed for the major portion of the inoculations because it is the species commonly found under storage-house conditions and seems to be responsible for most of the loss. *R. tritici* although not so common as *R. nigricans* is a very parasitic species, especially under artificial conditions. A comparison of these two species gives some idea of the results that may be expected from them, one requiring relatively low and the other one an intermediate temperature, or at least a temperature considerably above that recommended for the storage of sweet potatoes.

## METHODS OF EXPERIMENTATION

The susceptibility to infection and decay by *R. nigricans* was determined for the following varieties of sweet potatoes: Big Stem Jersey, Little Stem Jersey, Southern Queen, Porto Rico, Dooley, Georgia, Pierson, Dahomey, Triumph, Gold Skin, Haiti, Nancy Hall, Early Carolina, Yellow

[1] HARTER, L. L., WEIMER, J. L., and LAURITZEN, J. I. THE DECAY OF SWEET POTATOES (IPOMOEA BATATAS) PRODUCED BY DIFFERENT SPECIES OF RHIZOPUS. *In* Phytopathology, v. 11, no. 7, p. 279–284. Literature cited, p. 284. 1921.

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ielmont, and Red Brazil. The parasitism of *R. tritici* was determined or all the above-named varieties except the last four.

The mechanical operations involved in carrying out these experiments are identical with those previously employed [1] and will not be discussed here. The potatoes inoculated with *R. nigricans* and *R. tritici* were incubated at temperatures of from 20° to 22° and 30° C., respectively, these temperatures having been found in previous experiments to be favorable for the growth of these two organisms. Records of the percentage of infection and of the progress of decay were made at the end of 48 hours after inoculation and every day thereafter to the close of the experiment. The experiments were allowed to run for from four to six days.

## EXPERIMENTAL DATA

The results of the inoculations of the different varieties of sweet potatoes with *R. nigricans* are shown in the following table. The figures in columns 1 and 2 were obtained by taking an average of the results of the several experiments. The data presented in the second column are based on an estimation of the percentage of the total amount of decay when the experiments were terminated.

TABLE I.—*Percentage of sweet potatoes infected and the estimated percentage of decay at the end of the experiment*

| Variety. | Percentage of infection. | Percentage of decay at the end of the experiment. | Variety. | Percentage of infection. | Percentage of decay at the end of the experiment. |
|---|---|---|---|---|---|
| Porto Rico | 97 | 75 | Nancy Hall | 90 | 28 |
| Big Stem Jersey | 100 | 88 | Florida | 100 | 40 |
| Triumph | 97 | 60 | Red Brazil | 100 | 95 |
| Pierson | 97 | 73 | Haiti | 100 | 93 |
| Gold Skin | 100 | 100 | Dahomey | 97 | 53 |
| Little Stem Jersey | 100 | 98 | Southern Queen | 87 | 23 |
| Georgia | 90 | 93 | Yellow Belmont | 100 | 93 |
| Early Carolina | 100 | 95 | Dooley | 100 | 98 |

The results show that a large percentage of the potatoes became infectec by the method employed. There were cases, especially among the mor resistant varieties, where only a very small amount of the tissue abou the well decayed. In view of this fact it was frequently difficult to decid whether infection had actually taken place or whether the small amoun of decay was due to an enzym in the inoculum at the time the inocula tions were made. The writers finally decided to regard as infected a potatoes which had been softened for 1 cm. or more beyond the margi of the well. It is not unlikely that the percentage of infection of son of the varieties, especially those which resisted further decay, was act ally placed too high by this method.

[1] HARTER, L. L., WEIMER, J. L., and ADAMS, J. M. R. SWEET-POTATO STORAGE-ROTS. *In* Jour. A Research, v. 15, no. 6, p. 337–368, pl. 21–27. 1918. Literature cited, p. 366–368.
HARTER, L. L., WEIMER, L., and LAURITZEN, J. I. OP. CIT.

Although it was easy to estimate the relative amount of decay between very susceptible and very resistant varieties, it was more difficult to determine the percentage of total decay at the end of a given time. The percentage of the entire potato that would be decayed at the end of a certain number of days depended to a considerable extent on the size and shape of the potatoes. Naturally a larger percentage of a small sweet potato would be decayed in a given time than of a large one; also a short to nearly spherical potato would be completely decayed sooner than a long, cylindrical one. As to shape, the potatoes differed greatly. The potatoes of some varieties were short and chunky, while others were long. In view of these facts the writers wish to emphasize the danger of putting too much reliance on small differences. The results, however, show some wide differences which the authors believe to be a fair estimate of the relative susceptibility of the different varieties.

With respect to their susceptibility to decay by *R. nigricans*, the different varieties of sweet potatoes can be divided roughly into three groups as follows: 1, very susceptible; 2, quite resistant; and 3, intermediate. To the first belong Gold Skin, Little Stem Jersey, Georgia, Early Carolina, Red Brazil, Haiti, Yellow Belmont, and Dooley; to the third group belong Porto Rico, Big Stem Jersey, Triumph, Pierson, Florida, and Dahomey; and to the second, Nancy Hall and Southern Queen. As might be expected, a considerable variation exists between the different varieties of a single group. Furthermore, there is no sharp line of separation between the more resistant and the more susceptible varieties of two contiguous groups, the differences frequently being uo greater than that which exists between varieties of the same group. The Gold Skin is by far the most susceptible variety studied, decay being completed considerably in advance of that of any of the other varieties. The Little Stem Jersey is likewise very susceptible and with the iGold Skin stands out conspicuously as regards the rapidity with which t decays. The Big Stem Jersey, a variety grown extensively in the northern range of the sweet-potato belt, decays fairly rapidly and completely, and must be ranked high as a susceptible variety in the intermediate group. Nancy Hall and Southern Queen are the only representatives of the resistant group. Although the table shows a high percentage of infection, the amount of decay was always small, and within the limits of these experiments they must be regarded as much more resistant than any of the other varieties. The Florida is the only other variety that approximates these two in resisting decay by *R. nigricans*.

Although the object of these experiments was primarily to determine the susceptibility of the different commercial varieties of sweet potatoes to decay by *R. nigricans*, one single set of inoculations was made with *R. tritici*, using the same varieties with the exception of the four already mentioned. *R. tritici* was found to be parasitic on all the varieties. One outstanding fact as a result of these experiments seems to be that the Nancy Hall and Southern Queen, varieties which are especially resistant to *R. nigricans*, are rather susceptible to decay by *R. tritici*. A further comparison, therefore, was made of the relative parasitism of these two species on Nancy Hall and Southern Queen, using the Little Stem Jersey, a very susceptible variety, as a control.

After inoculation of the three varieties in the usual way with *R. tritici* and *R. nigricans* they were divided into two lots. One lot was in-

cubated at 30° C., a temperature favorable for *R. tritici*, and the other lot at 20° to 22°, a temperature favorable for the growth of *R. nigricans*. None of the potatoes inoculated with *R. nigricans* and incubated at 30° became infected, showing that this temperature is unfavorable to infection by this fungus. On the other hand, all those inoculated with *R. tritici* became infected and were completely decayed in three or four days. A direct comparison of these two organisms at 30° was therefore impossible. The percentage of infection as well as the total amount of decay of all three of these varieties by both organisms when incubated at a temperature of 20° to 22° for seven days is shown in Table II.

TABLE II.—*Percentage of infection and estimated percentage of decay of three varieties of sweet potatoes by R. nigricans and R. tritici after seven days at 20° to 22° C.*

| Variety. | Organism. | Percentage of infection. | Percentage of decay. |
|---|---|---|---|
| Southern Queen | *R. nigricans* | 70 | 15 |
| | *R. tritici* | 100 | 85 |
| Nancy Hall | *R. nigricans* | 80 | 40 |
| | *R. tritici* | 95 | 100 |
| Little Stem Jersey | *R. nigricans* | 100 | 80 |
| | *R. tritici* | 100 | 100 |

An examination of this table shows that at this temperature the Little Stem Jersey variety was decayed much more readily than either Nancy Hall or Southern Queen, by both organisms. However, decay was much more rapid by *R. tritici* than by *R. nigricans*, being completed by the former in three to four days and not quite completed by the latter in seven days. The potatoes of the Nancy Hall and Southern Queen varieties were 100 and 40 per cent and 85 and 15 per cent decayed by *R. tritici* and *R. nigricans*, respectively, at the end of seven days. A comparison of the three varieties shows that the estimated amount of decay caused by *R. nigricans* at the end of the experiment was about 15, 40, and 80 per cent for Southern Queen, Nancy Hall, and Little Stem Jersey, respectively. The total amount of decay caused by *R. tritici*, on the other hand, was 85, 100, and 100 per cent, respectively. The results therefore show that the Little Stem Jersey is quite susceptible to decay by both species, while Nancy Hall and Southern Queen are very resistant to decay by *R. nigricans* only. The percentage of infection of the Nancy Hall potatoes was 95 and 80 by *R. tritici* and *R. nigricans*, respectively, while the Southern Queen potatoes were 100 and 70 per cent infected by the same organisms. On the other hand, Little Stem Jersey potatoes were 100 per cent infected by both species.

These results indicate what occurs at temperatures of 20° and 30° C. under the conditions existing in these particular experiments. A temperature of 30° proved to be too high for *R. nigricans* and 20° to 22°, while not optimum for either organism, is probably more favorable for decay by *R. tritici* than by *R. nigricans*. On the other hand, if a much lower temperature had been employed it would have been more favorable to *R. nigricans* but less favorable to *R. tritici*. The results, however, do show a considerable difference in the three varieties with respect to their relative susceptibility to decay by the two species of Rhizopus at the temperatures tried.

## SUMMARY

(1) *R. nigricans* is parasitic on the following varieties of sweet potatoes: Porto Rico, Big Stem Jersey, Triumph, Pierson, Gold Skin, Little Stem Jersey, Georgia, Early Carolina, Nancy Hall, Florida, Red Brazil, Haiti, Dahomey, Southern Queen, Yellow Belmont, and Dooley. *R. tritici* was found to be parasitic on all the above-named varieties except Early Carolina, Florida, Red Brazil, and Yellow Belmont, on which it was not tried.

(2) With respect to their susceptibility to decay by these two species of Rhizopus, the varieties can be separated roughly into three groups—first, those that are very susceptible; second, those that are very resistant; and, third, those that are intermediate between the first and second. To the first group belong the Gold Skin, Little Stem Jersey, Georgia, Early Carolina, Red Brazil, Haiti, Yellow Belmont, and Dooley; to the second, Southern Queen and Nancy Hall; and to the third, Porto Rico, Big Stem Jersey, Triumph, Pierson, Florida, and Dahomey.

(3) Nancy Hall and Southern Queen, the two most resistant varieties, were more susceptible to decay by *R. tritici* than by *R. nigricans* at a temperature of from 20° to 22° C. Neither of these varieties, however, decayed as readily as the Little Stem Jersey under similar conditions and used as a control.

(4) The results of these experiments show that all the varieties tried (16) are more or less susceptible to decay by *R. nigricans* but that there are some varietal differences. Likewise, the results show that those varieties which decayed most readily under the conditions of these experiments are the ones which have been observed to decay most readily under commercial storage-house conditions.

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